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Genetic basis of variation for root traits and response to heat stress in durum wheat

Presentata da: Dr. Ghasemali Nazemi

Coordinatore Dottorato

Chiar.mo Prof. Giovanni Dinelli

Relatore

Prof. Silvio Salvi

Correlatore

Chiar.mo Prof. Roberto Tuberosa

Esame finale anno 2015

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Genetic basis of variation for root traits and response to heat stress in durum wheat

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Ghasemali Nazemi

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SUMMARY

Durum wheat is the second most important wheat species worldwide and the most important crop in several Mediterranean countries including Italy. Durum wheat is primarily grown under rainfed conditions where episodes of drought and heat stress are major factors limiting grain yield. The research presented in this thesis aimed at the identification of traits and genes that underlie root system architecture (RSA) and tolerance to heat stress in durum wheat, in order to eventually contribute to the genetic improvement of this species. The thesis describes and reports the results of four experiments.

In the first experiment, seedlings of 183 durum wheat elite accessions were evaluated in order to identify Quantitative Trait Loci (QTLs) using genome-wide association mapping for several RSA-related traits including root number, seminal root angle, primary root length, and others. Highly significant differences among accessions were detected for all traits. Out of the 48 QTLs detected, 15 overlapped with QTLs for agronomic traits and/or grain yield in two or more environments as detected in previous studies. Root number and seminal root angle appeared the most promising traits for further studies on the adaptive role of RSA plasticity on field performance in environments differing for water availability. Our results provide novel insights on the genetic control of RSA and its implications on field performance of durum wheat.

In the second experiment, the genetic basis of variation for RSA traits were investigated using a population of 176 recombinant inbred lines (RILs) derived from the cross between two Italian elite durum wheat (Meridiano and Claudio), in order to identify QTLs for RSA and compare their overlaps with other QTLs identified in other experiments and environments. The following seedling-stage RSA and seed traits were: root number, seminal root angle, primary root length, total root length, thousand kernel weight, shoot length, root and shoot dry weight. The results indicated a wide range of phenotypic variation for RSA traits. The largest heritability was observed for thousand kernel weight (78.6%) and seminal root angle (65.4%). In total, 48 novel QTLs for RSA traits were identified on all chromosomes, with the exception of chromosome 4A. Both parents contributed favorable alleles at QTLs. Among the considered RSA traits, seminal root angle appeared the most promising for undertaking further studies on the role of RSA traits. The most important QTLs for seminal root angle identified in this study mapped on chromosomes 4B and 6B.

Variation in root anatomical traits influences whole plant physiology and crop adaptation to adverse soil conditions and thus impacts yield and its stability. Typical components of anatomical root traits are the arrangement of cells and tissues as observed by microscopy sections. In the third experiment, we investigated the phenotypic variation of eleven root anatomical traits including

aerenchyma features in ten elite durum wheat cultivars. Significant differences among cultivars were identified for several traits. Trait heritability ranged from 0.12 (number of xylem vessels) to 0.72 (number of aerenchyma lacunae). While area and number of aerenchyma lacunae were highly correlated, neither trait correlated with other root features, suggesting an independent physiological and/or genetic control in respect to the other root anatomical traits. The old Italian founder cultivar Cappelli was shown to have a significantly higher portion of root aerenchyma of all the modern cultivars. These results show for the first time the presence of sizeable genetic variation in aerenchyma-related root anatomical traits in cultivated tetraploid wheats, prompting for additional studies aimed at mapping the quantitative trait loci governing such variation and to test their role in the adaptive response of durum wheat to abiotic stresses as related to soil conditions.

Heat stress is an agricultural problem in many areas of the world. In the fourth experiment, genome wide association mapping based on the panel of 183 elite of durum wheat accessions was deployed in order to dissect the genetic control and identify QTLs for response to heat stress. The experiment was conducted using a randomized complete block design with two replications in greenhouse environmental conditions. Cell membrane stability (CMS) was recorded as a proxy index to evaluate the response to heat stress in a three-step experiment: constitutive heat stress response, acquired heat stress response and constitutive-acquired heat stress response. Significant differences among genotypes were observed for all measured CMS traits. The highest heritability ($h^2 = 0.86$) was recorded for constitutive-acquired heat stress response. The panel was profiled with simple sequence repeat, Diversity Arrays Technology and sequence-tagged site markers (957 markers in total). Thirty four single marker/QTL regions were located in all chromosomes; four major QTLs (LOD $\geq$ 3) for constitutive heat stress response were detected on chromosome 5A, 6A, 7B, while one QTL for constitutive-acquired heat stress response was detected on chromosome 6B. The wide range of genetic variation and the limited influence of population structure support the reliability of our results and prompt for additional finer investigations of the physiological bases underlying these QTLs, towards their exploitation in breeding.

CHAPTER 1. INTRODUCTION

1.1 THE IMPORTANCE OF DURUM WHEAT

Wheat (*Triticum spp.*) is the most important stable food crop for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops (Shewry, 2009). It is grown worldwide on more than 219 million hectares, with a world production of 715 million tons in 2013 (http://faostat3.fao.org/). So, wheat is the third most-produced cereal after maize (well over 900 million tons) and rice (740 million tons), taking up more arable land than any other crop.

Durum wheat (*Triticum turgidum* L. var. *durum*, 2n = 4x = 28) is the second most important wheat species and the only tetraploid species of wheat of commercial importance that is widely cultivated today. It is an important crop for the agriculture and economy of Mediterranean countries where more than half of the global acreage of this crop is grown. Recently, this cereal has been the object of renewed interest, because of its valuable production and adaptation to low rainfall and semi-arid environments (Belaid, 2000; Morancho, 2000; Maccaferri et al. 2008; Habash et al. 2009). Durum wheat is primarily grown under rainfed conditions where the frequent occurrence of drought combined with heat stress is the major factor limiting grain yield (Araus et al. 2002, 2003a,b; Condon et al. 2004).

The major durum wheat producing countries are Italy, Spain and France in UE, Canada, Syria, USA, Algeria and Morocco, while minor production areas occur in Russia, Turkey, Tunisia, Mexico and India. Italy is the main market for the durum wheat production from the European Community. In addition, Italy has been conducting an intense breeding activity over the last century which has supported its long tradition of pasta making. Durum wheat has the ideal properties for making the best pasta. It is high in protein and gluten, both of which are necessary components for pasta making. Durum wheat production has been a part of people's diet, for a long time. The rapidly increasing demand for more durum wheat both in global and domestic markets, combined with the availability of proven technologies and practices in countries offer an excellent opportunity for commercialization of the crop so that the smallholder farmers can significantly participate in the production of high quality durum wheat to improve their income and livelihood (Newai 2006).

The durum wheat breeding program in Iran is conducted in collaboration with the International Centre for Agricultural Research in the Dry Areas (ICARDA) and is aimed at developing varieties

adapted to the different environments prevailing in Iran, including cold and mildly cold winter areas. The improved durum wheat genotypes are evaluated in multi-environment trials (MET) to test their performance across environments and to select the best genotypes in specific environments or stable performing genotypes across a range of environments. Genotype-by-environment (GxE) interaction is commonly encountered when different genotypes (cultivars) are evaluated in MET, as suggested by many studies/reports (Brancourt-Hulmel and Lecomte 2003; Yan and Kang 2003; Fan et al. 2007).

1.2 THE DURUM WHEAT GENOME

Among a rich variety of wheat species and forms, two are important for human diet, namely bread wheat (*Triticum aestivum*) and durum wheat (*Triticum turgidum*, i.e. *Triticum durum* Desf.). Bread and durum wheat are alloheaxaploid and allotetraploid species (see Fig. 2), with large genome size (16 and 11 Gb, respectively) rich in repetitive elements. All these features make wheat genome particularly complex among crops (Ganal and Röder 2007; Mayer et al. 2014).

Durum wheat, *Triticum turgidum* (Genome formula:AABB), evolved from the hybridization of two diploid ancestors. The A genome is considered as the pivot genome common to all wheat species and derives from an ancestor of the wild wheat *Triticum urartu* (AA genome, 2n = 14. Dvorak et al. 1992).The B genome likely originated from the SS genome of an *Aegilops* species belonging to the *Sitopsis* section and similar (Van Slageren 1994) to the present *Aegelops speltoides* (Sarkar and Stebbins 1956; Marcussen et al. 2014).

Bread wheat, *Triticum aestivum* (AABBDD), evolved from the second independent hybridization (Dubcovsky and Dvorak 2007; Marcussen et al. 2014) between an ancestor of the diploid *Aegilops tauschii* var. *strangulate* (DD genome, McFadden and Sears 1946) and an allotetraploid wheat. This hybridization occurred, probably, in the west of Iran 8,000 years ago, when the first cultivated tetraploid wheats (AABB) were introduced in the areas where the diploids wild wheats, holding the D genome, were already grown (Feldman and Sears 1981).

1.3 THE PROGRESSES IN DURUM WHEAT BREEDING

The first step in a breeding program consists in the creation of variability, usually by hybridization, with the aim of accumulating enough genetic variation and providing novel useful recombinant forms for the target traits in the progeny. The choice of parents for crosses requires a prioritization

of the goals to be achieved by breeding, and the collection and characterization of genetic sources carrying favourable alleles for the target traits (Royo et al. 2009).

Higher grain yield, shorter stature and early maturity are among the main breeding objectives in durum wheat. As it was stated, Italy is characterized by an intense breeding activity in the last century. During the first decades of the 20th century the breeding activities led to selection of several varieties from landraces grown in South Italy and from *africanum* (ie. from Africa) types of durum wheat (Bozzini 1970; D'Amato 1989). After the Second World War new breeding programs were developed to select durum wheat genotypes well adapted to the unfavorable environments of South Italy by introgressing useful traits from foreign *syropalestinian* types of durum wheat (i.e. Eiti) (De Cillis 1942). During the 1970s, a series of CIMMYT short straw recombinant lines were introduced in the Italian breeding programs (Bozzini et al. 1998). This strategy led to the release of new durum wheat cultivars with a high yield potential and high pasta making quality (Vallega and Zitelli 1973).

Comparisons of cultivars bred in different periods can shed an interesting light on the evolutionary trends in morphophysiological, agronomical and qualitative characteristics of the durum wheats grown in a given region and provide the most direct estimate of breeding progress (Jiang et al. 2003; Guarda et al. 2004; Shearman et al. 2005).

1.4 BREEDING FOR ABIOTIC STRESS TOLERANCE

The world population continues to increase rapidly and agriculture will have to increase its crop productivity by 70-110% in 2050 to feed the world (Tester and Langridge 2010; Tilman et al. 2011). This task is challenging, as not only we must increase crop yields by a margin not seen before but also we have to do this in a changing climate (Roy et al. 2011). Climate change associates with increased exposure to abiotic stress factors such as drought, heat and salt, all of which have major impacts on crop productions.

Genetic enhancement of crops is one of the most important strategies to increase productivity under less then optimal agricultural conditions. A key requirement of the wheat breeding programs is to develop varieties that can cope with a wide range of abiotic stresses while still maintaining high grain quality. Abiotic stress is an important consideration in wheat breeding, as plants need to be grown in a variety of locations and environmental conditions. They can influence crop growth, yield and quality of the desired product as well as contribute to the cost of production and to the use of pesticides and fertilizers.

In many areas that have been considered marginal for growing crops, due to their low fertility, drought, heat, salt, and other abiotic stresses typically act to disturb the production system. Additionally, climate change has brought new challenges to agriculture to produce food, feed, fiber and biofuels. To cope with these new challenges, many plant breeding programs have recently reoriented their breeding scope to stress tolerance. The tolerance to a particular stress is quite variable but is related to the plant's ability to withstand adverse conditions, survive, and reproduce successfully. Indeed, Miti et al. (2010) defined tolerance as the reduction in yield under stress conditions compared to the yield under the optimal condition of cultivation. The genetic control of abiotic stress tolerance is quantitative and involves many loci distributed in different regions of the genome in cultivated species (Wu et al. 2011).

1.4.1 Drought stress

Drought is the most significant environmental stress in agriculture worldwide and improving yield under drought is a major goal of plant breeding. Drought tolerance is defined as the ability of a plant to live, grow, and reproduce satisfactorily with limited water supply or under periodic conditions of water deficit (Turner 1979). In recent years, crop physiology and genomics have led to new insights in drought tolerance providing breeders with new knowledge and tools for plant improvement (Tuberosa and Salvi 2006).

Tolerance to drought is a complex quantitative trait controlled by several small effect genes or QTLs (Barnabas et al. 2008; Fleury et al. 2010). To address the complexity of plant responses to drought, it is vital to understand the physiological and genetic basis of this response. Failure to understand the molecular mechanisms of seed yield stability has hampered both traditional breeding and the use of modern genetics in the improvement of drought tolerance of crop plants (Passioura 2010; Sinclair 2011).

Drought stress is a primary limitation to crop production (Boyer 1982; Tuberosa et al. 2007), and important agroecosytems may face increasing drought risk as the result of global climate change (Trenberth et al. 2007). It is estimated that by 2025, over 60% of the human population will inhabit countries with water shortage (Arnel 1999). The identification and understanding of traits improving crop drought tolerance are essential for the development of more drought-tolerant crops and cropping systems. In the last decades, plant physiologists have identified a number of traits that might help plants adapt to drought, use acquired water efficiently and tolerate desiccation, as well as a smaller number of traits that may assist soil water acquisition (Blum 1996; Cattivelli et al.

2008; Sinclair et al. 1990; Bruce et al. 2002; Richards 2006; Nelson et al. 2007). For instance, root system architecture traits are the important regulator of water acquisition under drought. Plants with longer and deeper roots have better access to water resources available at depth, and are therefore more prevalent among species found in dry environments (Ehdaie et al. 2003; Manschadi et al. 2006, 2010; Asseng and Turner 2007; Lilley and Kirkegaard 2007; Hammer et al. 2009; Wasson et al. 2012; Uga et al. 2013). While plants generally allocate relatively more resources to the root system in response to mineral deficiencies and drought (Lynch, 2007a, b).

1.4.2 Heat stress

Heat stress adversely affects wheat production in many regions of the world and is particularly detrimental during reproductive development. Heat stress is defined as increased temperature level sufficient to cause irreversible damage to plant growth and development. Increasing the heat tolerance of crop species would therefore help to increase and stabilize crop production around the world. To this end, utilizing diverse genetic resources for breeding is a potentially important strategy.

Wheat growth and development is divided into three phases: vegetative, reproductive, and grain filling. Heat stress during the vegetative stage is not of major concern due to the sowing of wheat during the winter or spring months. Immediately prior to anthesis, the number of grains are determined and subsequently filled following anthesis. The number of grains and individual kernel weight make up the two major yield components in wheat (Satorre and Slafer 1999). Depending on the timing, intensity, and duration of heat stress, grain set and grain filling may be disrupted, therefore compromising yield.

Measurement of cell membrane stability (CMS) is a technique that has been used for as an indirect measure for both heat and drought tolerance in various crops (Sullivan 1972) and it is a tool already applied in breeding programs for heat tolerance (Ibrahim and Quick 2001a, b; Ottaviano et al. 1991; Tripathy et al. 2000).

CMS is a measure of electrolyte diffusion resulting from heat-induced cell membrane leakage (Blum and Ebercon 1981, Saadalla et al. 1990). The technique has already been used to screen and evaluate different wheat genotypes for thermal tolerance (Yildirim et al. 2009).

1.4.3 Salt stress

Salt stress is a major constraint to agricultural food production because it decreases crop yield and restricts the use of agricultural land. The problem is increasing annually due to climatic change and

poor irrigation management. Most cultivated crops are salt sensitive and therefore salinity is an ever-present threat to agriculture (Flowers and Flowers 2005). Salt tolerance in crop plants is a genetic and physiological complex trait and is controlled by several quantitative trait loci (Flowers 2004; Nguyen et al. 2013).

The plant response to salinity stress is composed of two phases (Munns and Tester 2008). The first phase concerns the osmotic stress that is perceived immediately upon plant exposure to highly saline conditions. Osmotic stress makes uptake of water by plants difficult and adversely affects shoot and root growth. To facilitate water uptake under such conditions, plants have to accumulate extra solutes to maintain the water balance of the cells. The second phase is manifested when high concentrations of toxic ions are built up over a longer period of time. As NaCl is a major constituent of saline soil, plants accumulate Na^+ and Cl^- ions up to levels that are toxic, reducing amongst others their photosynthetic capacity (Tavakkoli et al. 2011). Therefore, both shoot Na^+ and Cl^- contents were considered important factors for salt-induced damage (Hasegawa et al. 2000; Munns and Testers 2008; Teakle and Tyerman 2010) even more because the toxicity effects of these ions appear to be cumulative (Tavakkoli et al. 2011).

1.5 THE STUDY OF ROOT SYSTEM ARCHITECTURE

Root system architecture (RSA) has emerged in recent years as an important focus for plant genetics and breeding study (Smith and De Smet et al. 2012, Orman-Ligeza et al. 2013). The key impediment to genetic analysis of cereal root system architecture has so far been the ability to study roots *in situ*. Howeverprotocols streamlining phenotypic observations have now been developed for adult plants under field conditions (Manschadi et al. 2006; Wojciechowski et al. 2009; Trachsel et al. 2011) and, more frequently, for young and/or adult plants grown in rizothrons under controlled environmental conditions (Bengough et al. 2004; Sanguineti et al. 2007; Liu et al. 2013). Analyses of genetic factors contributing to root system architecture remain however limited, partly because of the above noted difficulty of observing the distribution of roots in field conditions, and partly because of the complexity of the effects of environmental conditions on root system architecture.

Throughout its life cycle, root system architecture (RSA) is finely tuned to the requirements of the whole plant. Roots play several essential roles, including anchoring to the soil, mechanical support to stems, uptake of water and nutrients (therefore playing an essential role in environmental stress tolerance) and others (De Dorlodot et al. 2007; Osmont et al. 2007; Smith and De Smet 2012)**.** Root phenotypic traits are thus characterized by plasticity because roots are organs which primarily respond to water and nutrient availability levels in the soil (Grossman and Rice, 2012). However,

root system architecture traits are also characterized by constitutive genetic inheritance components which allows to predict the root phenotypes at the adult plant stage based on observations carried out at seedling stage, which is much more manageable.

In this context, the study of root architectural system (RSA) features/QTLs as related to crop performance can help identifying proxy traits for enhancing adaptation to different soil properties, moisture conditions, nutrient concentration, etc. (Bacon et al. 2003; Yu et al. 2007; Hochholdinger and Tuberosa 2009; Obara et al. 2010; Tuberosa 2013; Uga et al. 2013; Lynch 2013). For example, deep roots might provide a higher protection against dehydration by extracting water stored in deeper soil horizons (Ehdaie et al. 2003; Manschadi et al. 2006, 2010; Asseng and Turner 2007; Hammer et al. 2009; Lilley and Kirkegaard 2011; Wasson et al. 2012; Uga et al. 2013). Therefore, identifying and introgressing alleles for deeper rooting in shallow-rooted, drought-susceptible cultivars (Grando and Ceccarelli 1995; Ehdaie et al. 2010; Steele et al. 2007; Uga et al. 2013) appears a desirable approach, as underlined by the "steep, cheap and deep" ideotype recently proposed by Lynch (2013).

1.6 MOLECULAR MARKERS AND THEIR APPLICATIONS IN BREEDING

Molecular marker technologies offer a wide range of novel approaches to improve the efficiency of selection strategies. Broadly explained, molecular marker technologies are based on the detection of sequence variation between varieties where the sequence variant sits in a region of the genome closely linked to a trait of interest.

Crop improvement relies on the effective utilization of genetic diversity. Molecular marker technologies promise to increase the efficiency of managing genetic diversity in breeding programmes. DNA-based molecular markers have several advantages over the traditional phenotypic selection and their potential benefits as marker-assisted selection (MAS) have been widely discussed (Melchinger, 1990; Paterson et al. 1991; Young 1996; Mohan et al. 1997; Anderson 2003; Varshney and Tuberosa 2007), especially to provide solutions to overcome some of the problems faced by classical phenotypic screening approaches in plant breeding programs.

The most widely used systems, adopted at different stages in the evolution of marker technologies, are restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) (Botstein et al. 1980; Weber and May 1989; Williams et al. 1990; Vos et al. 1995; Chee et al. 1996). These technologies can genotype

agricultural crops with varying degrees of efficiency. They have various degrees of limitations associated with their capability to quickly develop and/or rapidly assay large numbers of markers. Although some of these limitations can be alleviated by equipment (e.g. highly parallel capillary electrophoresis), most of them are inherently linked to the sequential nature, low reproducibility, or high assay costs of the marker technologies, or the reliance on DNA sequence information. Diversity arrays technology (DArT) was developed as a hybridisation-based alternative, which captures the value of the parallel nature of the microarray platform (Jaccoud et al. 2001; Akbari et al. 2006).

1.7 IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL)

Quantitative trait loci (QTLs) are loci which segregation in an experimental of natural population affect the expression of a quantitative trait (Long et al. 2008). A QTL is generally identified with the help of moleculargenetic markers. Recent technical advancements and refinement of analytical methods are making QTL mapping easier and more popular (Salvi and Tuberosa 2005; Salvi and Tuberosa 2015). Several methods have been developed for the identification of QTLs. The most basic is the use of analysis of variance to compare the score of the quantitative trait between each marker allele (Soller et al. 1976; Zeng 1994). Although easy to carry out, this method has several severe drawbacks: it requires a high population of samples, it cannot distinguish between multiple QTLs on the same chromosome or marker, and it cannot describe the probable position of the QTL on the chromosome.

A more powerful method for QTL detection is interval mapping. Interval mapping uses multiple markers to assign a likelihood profile to each region of the chromosome. The individual regions are defined as intervals between two markers, with the strength of the correlation between each set of markers and the score of the quantitative trait being used to assign the likelihood profile. Regions with a likelihood profile greater than a pre-assigned threshold are designated as QTL. This technique is superior to analysis of variance as it gives the probable location of the QTL and requires fewer samples. However, it has a similar degree of difficulty in distinguishing between multiple QTLs on the same chromosome (Lander and Botstein 1989; Zeng 1994). To ameliorate this problem a technique called Composite Interval Mapping (CIM) may be used. Using CIM it is possible to distinguish between separate QTLs on the same chromosome by assigning groups of markers as a proxy for the already established QTL. The effect of these QTL proxy markers is then taken into account when calculating likelihood profiles for other regions on the chromosome (Zeng

1994). Composite interval mapping aids in distinguishing between multiple QTLs on the same chromosome but is dependent on researcher's accuracy in designating the markers to serve as QTL proxies.

1.7.1 Use of Segregating Populations

QTL mapping can be carried out in segregating populations such as Recombinant inbred lines (RILs), Near isogenic lines (NIL), or Double haploid lines (DH). Recombinant inbred lines (RILs) are developed by crossing two inbred parent lines followed by repeated selfing to create a new inbred line, whose genome is a mosaic of the parental genomes. As each RIL is an inbred strain, it can be propagated eternally and can be used for genetic mapping. The progeny are allowed to self-fertilize until homozygosity is achieved and the lines are then used to identify QTLs related to the phenotypic differences between the parents (Browman 2004).

Another type of very valuable permanent population in genetic mapping of target traits is represented by near isogenic lines (NILs). Such genetic stocks are characterized by an isogenic background with the exception of single chromosome regions (ideally one per line) introgressed (substituted) from a donor accessions. They are usually generated though back-crossing to a recurrent parent at least for six generations, in order to be able to selectively analyse the phenotypic effect attributable to a QTL (Pumphrey et al. 2007; Xu and Crouch 2008). The introgressed chromosome regions may carry the QTL and could therefore drive to an hypothesis-driven high-resolution mapping.

Diploids produced from chromosome doubling of haploids are called doubled or double haploid (DH). The DH approach has several advantages that make it useful in genetics and plant breeding. Forster et al. (2007) reviewed various approaches for haploid production in plants. Forster and Thomas (2004) and Szarejko and Forster (2007) reviewed the use of DHs in genetic studies and plant breeding. DHs have been used in plant breeding programmes to produce homozygous genotypes in a number of important species, e.g. tobacco (*Nicotiana tabacum* L.), wheat, barley, canola (*Brassica napus* L.), rice and maize (Maluszynski et al. 2003)

DH populations are desirable genetic materials for genetic mapping including the construction of genetic linkage maps and gene tagging using genetic markers. QTL analysis is facilitated by using DH mapping populations and the homozygosity of DHs enables accurate phenotyping by replicate trials at multiple sites (Forster and Thomas 2004).

1.7.2 Germplasm collection and association mapping approaches

Association mapping (AM) or linkage disequilibrium (LD) approach is receiving increasing attention as QTL mapping method complementary to biparental mapping populations. AM seeks a phenotype-locus association in populations of unrelated genotypes. AM has recently been advocated as the method of choice for identifying loci involved in the inheritance of complex traits in plant genetic research as well (Flint-Garcia et al. 2003; Kraakman et al. 2006; Cockram et al. 2010; Zhao et al. 2007; Atwell et al. 2010; Kloth et al. 2012) and it has been demonstrated to be promising to exploit the full potential of novel molecular marker and sequencing technologies (Zhu et al. 2008).

Besides basic plant and cell biology, however mapping individual QTLs is only useful inasmuch as QTLs are transferrable to other populations of the same or related crop species (Collard et al. 2005). Association mapping relies on the presence of trait-associated linkage disequilibria in collections of widely diverse germplasm (Mackay and Powell 2007).

AM can achieve a higher resolution of causal trait polymorphism than linkage mapping. In addition AM can also accommodate germplasm with broader genetic variation (i.e. from breeding lines to landraces and even wild progenitors) and allows for the mapping of many traits simultaneously. Thus, there is reduced need to develop expensive and time-consuming biparental populations for each target trait. However, because of the much reduced LD extent in AM populations compared to linkage mapping populations, a significantly greater number of genetic markers are needed to cover the whole genome and perform a genome-wide association scan (Nordborg and Weigel 2008; Neuman et al. 2010). With the number of available robust genetic markers such as SSRs and Single Nucleotide Polymorphisms (SNPs) increasing and the cost of genotyping decreasing, AM has become a more attractive approach for revealing the genetic architecture of various traits in crop species

Generally, AM involves six steps (see Fig. 3 from Al-Maskri et al. 2012): (1) selection of a diverse association panel/group of individuals from a natural population/germplasm collection that may include, land races, elite cultivars, wild relatives and exotic accessions (2) a comprehensive and precise phenotyping is performed over the traits such as yield, stress tolerance or quality related traits of the selected genotypes in multiple repeats and years/environments, (3) the genotypes are then scanned with suitable molecular markers (AFLP, SSRs, SNPs), (4) population structure and kinships are determined to avoid false positives followed by (5) quantification of LD extent using different statistics like D, D' or r^2. Finally, (6) genotypic and phenotypic data are correlated using appropriate statistical software allowing tagging of molecular marker positioned in close proximity

of gene(s) underlying a specific trait. Consequently, the tagged gene can be mobilized between different genotypes and/or cloned and annotated for a precise biological function.

Recently, several AM studies have been published on a variety of crops including common wheat (Breseghello and Sorrells 2006; Ravel et al. 2006; Roy et al. 2006; Crossa et al. 2007; Jing et al. 2007; Tommasini et al. 2007; Peng et al. 2008; Liu et al. 2013), durum wheat (Sanguineti et al. 2007; Maccaferri et al. 2010; 2011), barley (Kraakman et al. 2004; Kraakman et al. 2006; Rostoks et al. 2006; Cockram et al. 2008; Varshney et al. 2012), maize (Remington et al. 2001; Wilson et al. 2004; Weber et al. 2007; Lua et al. 2010), and rice (Agrama et al. 2007).

1.7.3 Marker assisted selection

In marker-assisted selection (MAS) breeding, the plant breeder takes advantage of the association between agronomic traits and allelic variants of genetic, mostly molecular, markers. The general idea behind marker-assisted breeding is as follows. Before a breeder can utilize linkage-based associations between traits and markers, the associations have to be assessed with a certain degree of accuracy and thus marker genotypes can be used as indicators or predictors of trait genotypes and phenotypes. When the alleles in question are few in number and have major effects on phenotype, such as a single gene based disease resistance, mapping a monogenic trait goes along with the mapping of markers, while introduction of the desired alleles into the cultivar can be carried out readily by the classical breeding procedures of crossing, backcrossing, selfing and selection. In both cases, breeders depend on a clear relationship between genotype and phenotype to monitor the presence of the desired alleles in the populations of concern. For quantitative traits, however, a reliable assessment of trait–marker association requires large scale field experiments as well as statistical techniques. One of the potential outcome is to allow the breeder to monitor the transmission of trait genes via closely linked markers, thus enabling ‘genotype building’, or ‘haplotype breeding’ (Salvi and Tuberosa 2015) i.e. construction of desired genotypes by deliberate crossing and selection, using the marker genotype as a selection criterion.

Several quantitative loci are often detected as associated with a complex phenotype and, as a consequence of the high LD encountered in experimental crosses, markers can reliable act as predicting variables (Whittaker et al. 1995). Some authors (e.g. Hospital et al. 1997) underlined that the possible fixation of unfavourable alleles at loci with small effect on the phenotype (often undetected in QTL mapping) could significantly affect the efficiency of MAS over breeding cycles.

1.8 REFERENCES

Agrama HA, Eizenga GC, Yan W (2007) Association mapping of yield and its components in rice cultivars. Mol. Breed. 19:341-356

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Al-Maskri AY, Sajjad M, Khan SH (2012) Association mapping: a step forward to discovering new alleles for crop improvement. Int. J. Agric. Biol. 14: 153-160

Anderson JA (2003) Plant genomics and its impact on wheat breeding. In: Plant molecular breeding (H.J.Newbury, Ed.), Blackwell Publ., Boca Raton, 184-215

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C-3 cereals: What should we breed for? Ann. Bot. 89: 925–940

Araus JL, Bort J, Steduto P, Villegas D, Royo C (2003a) Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. Ann. Appl. Biol. 142: 129–141

Araus JL, Villegas D, Aparicio N, Del Moral LF, El Hani S, Rharrabti Y, Ferrio JP, Royo C (2003b) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci. 43: 170–180

Arnel NW (1999) Climate change and global water resources. Global Environmental Change 9, 31–49

Asseng S, Turner NC (2007). Modeling genotype x environment x management interactions to improve yield, water use efficiency and grain protein in wheat. In 'Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations.Vol 21.' (Eds JHJ Spiertz, PC Struik and HH VanLaar) pp. 93-103

Atwell S, Huang YS, Vilhjalmsson BJ, Willems G, Horton M, Li Y, Meng D, Platt A, Tarone AM, Hu TT, Jiang R, Muliyati NW, Zhang X, Amer MA, Baxter I, Brachi B, Chory J, Dean C, Debieu M, de Meaux J, Ecker JR, Faure N, Kniskern JM, Jones JD, Michael T, Nemri A, Roux F, Salt DE, Tang C, Todesco M, Traw MB, Weigel D, Marjoram P, Borevitz JO, Bergelson J, Nordborg M (2010) Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465:627-631

Bacon MA, Davies WJ, Mingo D, Wilkinson S (2003) Root signals. Roots: the hidden half. Marcel Dekker Inc, New York, pp 460–471

Barnabas B, Jager K, Feher A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 31:11–38

Belaid A (2000) Durum wheat in WANA: production, trade and gains from technological change. Proceedings of the seminar 'Durum wheat improvement in the Mediterranean region: new challenges. Zaragoza, Spain, 12–14 April 2000. Options Mediterraneennes, Serie A, Seminaires Mediterraneens, 40, 35–49

Bengough AG, Gordon DC, Al-Menaie H, Ellis RP, Allan D, Keith R, Thomas WTB, Forster BP, (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant and Soil, 262: 63–70

Blum A (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation 20: 135–148

Blum A, Ebercon A (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science 21: 43-47

Botstein D, White R, Skolnick M, Davis R (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Boyer JS (1982) Plant productivity and environment. Science 218: 443–448

Bozzini A (1970) Genetica e miglioramento genetico dei frumenti duri. Genet. Agr. 24, 145–193.

Bozzini A, Corazza L, D'Egidio MG, Di Fonzo N, La Fiandra D, Pogna NE, Poma I, (1998) Durum wheat (Triticum turgidum spp. durum). In: Scarascia Mugnozza, G.T., Pagnotta, M.A. (Eds.), Italian Contribution to Plant Genetics and Breeding. Viterbo, Italy, pp. 181–194

Brancourt-Hulmel M, Lecomte C (2003) Effect of environmental variates on genotype 3 environment interaction of winter wheat: A comparison of biadditive factorial regression to AMMI. Crop Science 43, 608–617

Breseghello F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat (Triticum aestivum L.) cultivars. Genetics 172:1165-1177

Browman KW (2004) "The Genomes of Recombinant Inbred Lines", Genetics 169:1133-1146.

Bruce W., Edmeades G. & Barker T. (2002) Molecular and physiological approaches to maize improvement for drought tolerance. Journal of Experimental Botany 53:13–25

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Mare C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Research 105, 1–14

Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SPA (1996) Accessing genetic information with high-density DNA arrays. Science

274:610–614

Cockram J, White J, Leigh FJ, Lea VJ, Chiapparino E, Laurie DA, Mackay IJ, Powell W, O'Sullivan DM (2008) Association mapping of partitioning loci in barley. BMC Genetics 9(16). doi: 10.1186/1471-2156-9-16

Cockram J, White J, Zuluaga DL, Smith D, Comadran J, Macaulay M, Luo ZW, Kearsey MJ, Werner P, Harrap D, Tapsell C, Liu H, Hedley PE, Stein N, Schulte D, Steuernagel B, Marshall DF, Thomas WTB, Ramsay L, Mackay I, Balding DJ, Waugh R, O'Sullivan DM, Consortium A (2010) Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. Proceedings of the National Academy of Sciences of the United States of America 107:21611-21616

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-196

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. J. Exp. Bot. 55: 2447–2460

Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH and Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177: 1889-1913

D'Amato F (1989) The progress of Italian wheat production in the first half of the 20th century: the contribution of breeders. Agr. Med. 119, 157–174

De Cillis U (1942) In: Maimone, G. (Ed.), The sicilian wheats (in Italian), pp. 1–323

De Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends in plant science, 12(10), 474-481

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. Science, 316: 1862-1866

Dvorak J, Akhunov ED, Akhunov AR, Deal KR, Luo MC (2006) Molecular characterization of a diagnostic DNA marker for domesticated tetraploid wheat provides evidence for gene flow from wild tetraploid wheat to hexa-ploid wheat. Mol Biol Evol, 23: 1386-1396

Ehdaie B, Whitkus RW, Waines JG (2003) Root biomass, water use efficiency, and performance of wheat-rye translocations of chromosomes 1 and 2 in spring bread wheat 'Pavon'. Crop Sci 43:710–717

Ehdaie B, Merhaut DJ, Ahmadian S, Hoops AC, Khuong T, Layne AP, Waines JG (2010) Root system size influences water-nutrient uptake and nitrate leaching potential in wheat. J Agron Crop Sci 196:455–466. doi:10.1111/j.1439-037X.2010.00433.x

Fan XM, Kang MS, Chen H, Zhang Y, Tan J, Xu C (2007) Yield stability of maize hybrids evaluated in multi-environment trials in Yunnan, China. Agronomy Journal 99, 220–228

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. J Exp Bot. 61:3211–3222

Flint-Garcia SA, Thornsberry JM, Buckler ESt (2003) Structure of linkage disequilibrium in plants. Annu Rev Plant Biol 54:357-374

Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55:307–319

Flowers TJ, Flowers SA (2005) Why does salinity pose such a difficult problem for plant breeders? Agric Water Manag 78:15–24

Forster BP, Thomas WTB (2004) Doubled haploids in genetics and plant breeding. Plant Breeding Reviews 25, 57–88

Forster BP, Herberle-Bors E, Kasha KJ, Touraev A (2007) The resurgence of haploids in higher plants. Trends in Plant Science 12, 368–375

Ganal MW, Roder MS (2007) Microsatellite and SNP markers in wheat breeding, Vol. 2. In: Varshney RK, Tuberosa R, (Eds.), Genomic assisted crop improvement: genomics applications in crops. The Netherlands: Springer, 1-24

Grando S, Ceccarelli S (1995) Seminal root morphology and coleoptile length in wild (Hordeum vulgare ssp. spontaneum) and cultivated (Hordeum vulgare ssp. vulgare) barley. Euphytica 86:73–80

Grossman JD, Rice KJ (2012) Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. Evol Appl 5:850–857. doi:10.1111/j.1752-4571.2012.00263.x

Guarda G, Padovan S, Delogu G (2004) Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. Eur. J. Agron. 21, 181–192

Habash DZ, Kehel Z, Nachit M (2009) Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J Exp Bot 60: 2805–2815

Hammer GL, Dong ZS, McLean G, Doherty A, Messina C, Schusler J, Zinselmeier C, Paszkiewicz S, Cooper M (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the US Corn Belt? Crop Sci 49:299–312. doi:10.2135/cropsci2008.03.0152

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Phys 51:463–499

Hochholdinger F, Tuberosa R (2009) Genetic and genomic dissection of maize root development and architecture. Curr Opin Plant Biol 12:172–177. doi:10.1016/j.pbi.2008.12.002

Hospital F, Moreau L, Lacoudre F, Charcosset A, Gallais A (1997) More on the efficiency of marker-assisted selection. Theoretical and Applied Genetics, 95: 1181-1189

Ibrahim A, Quick JS (2001a) Heritability of heat tolerance in winter and spring wheat. Crop Sci. 41:1401–1405

Ibrahim A, Quick JS (2001b) Genetic control of high temperature tolerance in wheat as measured by membrane thermal stability. Crop Sci. 41:1405–1407

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:e25

Jiang, GM, Sun JZ, Liu HQ, Qu CM, Wang KJ, Guo RJ, Bai KZ, Gao LM, Kuang TY (2003) Changes in the rate of photosynthesis accompanying the yield increase in wheat cultivars released in the past 50 years. J. Plant Res. 116, 347–354

Jing HC, Kornyukhin D, Kanyuka K, Orford S, Zlatska A, Mitrofanova OP, Koebner R, Hammond-Kosack K (2007) Identification of variation in adaptively important traits and genome-wide analysis of trait-marker associations in Triticum monococcum. Journal of Experimental Botany 58: 3749-3764

Kloth KJ, Thoen MPM, Bouwmeester HJ, Jongsma MA, Dicke M (2012) Association mapping of plant resistance to insects. Trends in Plant Science 17:311-319

Kraakman ATW, Niks RE, Van den Berg PMM, Stam P, Van Eeuwijk FA (2004) Linkage Disequilibrium Mapping of Yield and Yield Stability in Modern Spring Barley Cultivars. Genetics 168: 435-446

Kraakman ATW, Martinez F, Mussiraliev B, van Eeuwijk FA, Niks RE (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Molecular Breeding 17:41-58

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199

Lilley JM, Kirkegaard JA (2007) Seasonal variation in the value of subsoil water to wheat: simulation studies in southern New South Wales. Aust J Agric Res 58:1115–1128. doi:10. 1071/ar07046

Liu WX, Danivola TV, Rouse MN, Bowden RL, Friebe B, Gill BS, Pumphrey MO (2013)

Development and characterization of a compensating wheat-Thinopyrum intermedium Robertsonian translocation with Sr44 resistance to stem rust (Ug99). Theor Appl Genet: 126(5):1167-77

Liu X, Li R, Chang X, Jing R (2013) Mapping QTLs for seedling root traits in a doubled haploid wheat population under different water regimes. Euphytica: 189: 51–66

Long Y, Zhang C, Meng J (2008) Challenges for QTL analysis in crops. J. Crop Sci. Biotech. 11 (1): 7 ~ 12

Lua Y, Zhangc S, Shah T, Xiec C, Haoc Z, Lic X, Farkharib M, Ribaut JM, Caoa M, Ronga T, Xu Y (2010) Joint linkage–linkage disequilibrium mapping is a powerful approach to detecting quantitative trait loci underlying drought tolerance in maize. Proc Natl Acad Sci USA. 107:19585–19590. doi: 10.1073/pnas.1006105107

Lynch JP (2007a) Rhizoeconomics: the roots of shoot growth limitations. Hortscience 42: 1107–1109

Lynch JP (2007b) Roots of the second green revolution. Australian Journal of Botany 55: 493–512

Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357. doi:10.1093/aob/mcs293

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Ben Salem M, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178: 489-511

Maccaferri M, Sanguineti MC, Mantovani P, Demontis A, Massi A, Ammar K, Kolmer J, Czembor J, Ezrati S, Tuberosa R (2010) Association mapping of leaf rust response in durum wheat. Mol Breed 26: 189-228

Maccaferri M, Sanguineti MC, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2011) Association mapping in durum wheat grown across a broad range of water regimes and yield potential. J Exp Bot 62: 409-438

Mackay I, Powell W (2007) Methods for linkage disequilibrium mapping in crops. Trends in Plant Science 12:57-63

Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) (2003) Doubled Haploid Production in Crop Plants: a Manual. Kluwer Academic Publishers, Dordrecht, Netherlands

Manschadi AM, Christopher J, Devoil P, Hammer GL (2006). The role of root architectural traits in

adaptation of wheat to water-limited environments. Funct Plant Biol 33:823–837

Manschadi AM, Christopher JT, Hammer GL, Devoil P (2010) Experimental andmodelling studies of drought-adaptive root architectural traits in wheat (Triticum aestivum L.). Plant Biosyst 144:458–462. doi:10.1080/11263501003731805

McFadden ES, Sears ER (1946) The origin of Triticum spelta and its free-threshing hexaploid relatives. Journal of. Heredity, 37: 81-89, 107-116

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104: 1-19

Miti F, Tongoona P, Derera J (2010) S1 selection of local maize landraces for low soil nitrogen tolerance in Zambia. Afr J Plant Sci 4:67–81

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker assisted selection in crop plants. Mol Breed 3:87-93

Morancho J (2000) Production and marketing of durum wheat worldwide. Proceedings of the seminar 'Durum wheat improvement in the Mediterranean region: new challenges. Zaragoza, Spain, 12–14 April 2000. Options Mediterraneennes, Serie A, Seminaires Mediterraneens, 40, 35–49

Marcussen et al. (2014) Ancient hybridizations among the ancestral genomes of bread wheat. International Wheat Genome Sequencing Consortium (IWGSC). Science, 18;345(6194):1250092

Mayer KFX et al. (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. International Wheat Genome Sequencing Consortium (IWGSC). Science, 18;345(6194):1251788

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nelson DE, Repetti PP, Adams TR, et al. (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. Proceedings of the National Academy of Sciences of the United States of America 104: 16450–16455

Neumann K, Kobiljski B, Dencic S, Varshney RK and Borner A (2010) Genome wide association mapping: A case study in bread wheat (Triticum aestivum L.). Molecular breeding. DOI 10.1007/s11032-010-9411-7

Newai Gebre-ab (2006) Commercialization of smallholder agriculture in Ethiopia. Ethiopian Development Research Institute, Addis Ababa. 22pp

Nguyen V, Ribot S, Dolstra O, Niks R, Visser RF, Linden CG (2013) Identification of quantitative trait loci for ion homeostasis and salt tolerance in barley (Hordeum vulgare L.). Mol Breed

31(1):137–152. doi:10.1007/s11032-012-9777-9

Nordborg M, Weigel D (2008) Next-generation genetics in plants. Nature 456: 720-723

Obara M, Tamura W, Ebitani T, Yano M, Sato T, Yamaya T (2010) Fine-mapping of qRL6.1, a major QTL for root length of rice seedlings grown under a wide range of $NH4^{(+)}$ concentrations in hydroponic conditions. Theor Appl Genet 121:535–547. doi:10.1007/s00122-010-1328-3

Orman-Ligeza B, Parizot B, Gantet PP, Beeckman T, Bennett MJ, Draye X (2013) Post-embryonic root organogenesis in cereals: branching out from model plants. Trends Plant Sci. 18: 459–467

Ottaviano E, Gorla MS, Pe E, Frova C (1991) Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. Theor. Appl. Genet. 81:713–719

Passioura JB (2010) Scaling up: the essence of effective agricultural research. Funct Plant Biol. 37:585–591

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv. Agron. 46:39-90

Peng JH, Bai Y, Haley SD, Lapitan NLV (2008) Microsatellite-based molecular diversity of bread wheat germplasm and association mapping of wheat resistance to the Russian wheat aphid. Genetica 135 (1): 95-122

Pumphrey MO, Bernardo R, Anderson JA (2007) Validating the Fhb1 QTL for Fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. Crop Science, 47: 200-206

Ravel C, Praud S, Murigneux A, Linossier L, Dardevet M, Balfourier F, Dufour P, Brunel D, Charmet G (2006) Identification of Glu-B1-1 as a candidate gene for the quantity of high-molecular-weight glutenin in bread wheat (Triticum aestivum L.) by means of an association study. Theor Appl Genet 112: 738-743

Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich Goodman MM, Buckler ES (2001) Structure of linkage disequilibrium and phenotypic associations in the maize genome. PNAS 98: 11479-11484

Richards R (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management 80:197–211

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney R K, Marshall DF, Graner A, Close TJ, Waugh R (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. PNAS 103: 18656-18661

Roy JK, Bandopadhyay R, Rustgi S, Balyan HS, Gupta PK (2006) Association analysis of

agronomically important traits using SSR, SAMPL, and AFLP markers in bread wheat. Current Science 90: 683-689

Roy SJ, Tucker EJ, Tester M (2011) Genetic analysis of abiotic stress tolerance in crops. Current Opinion in Plant Biology 14:232-239

Royo C, Elias ME, Manthey FA (2009) Durum wheat breeding. M.J. Carena (ed.), Cereals, DOI:10.1007/978/-0-387-72297-9

Saadalla MM, Shanahan JF, Quick JS (1990) Heat tolerance in winter wheat: I. hardening and genetic effects on membrane thermostability. Crop Science 30:1243-1247

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10(6):297–304

Salvi S, Tuberosa R (2015) The crop QTLome comes of age. Curr Opin Biotechnol. 19;32C:179-185

Sanguineti MC, Li S, Maccaferri M, Corneti S, Rotondo F, Chiari T, Tuberosa R 2007. Genetic dissection of seminal root architecture in elite durum wheat germplasm. Ann Appl Biol 151: 291-305

Sarkar P, Stebbins GL (1956) Morphological evidence concerning the origin of the B genome in wheat. American Journal of Botany, 43: 297-304

Satorre EH, Slafer GA (1999) Wheat: ecology and physiology of yield determination. The Hawthorn Press ISBN 1-56022-874-1, Stroud, Gloucestershire, UK

Shearman VJ, Sylvester-Bradley R, Scott RK, Foulkes MJ (2005) Physiological processes associated with wheat yield progress in the UK. Crop Sci. 45, 175–185

Shewry PR (2009) The HEALTHGRAIN programme opens new opportunities for improving wheat for nutrition and health. Nutrition Bulletin, 34(2): 225–231

Sinclair TR, Bennett JM, Muchow RC (1990) Relative sensitivity of grain yield and biomass accumulation to drought in field-grown maize. Crop Science 30, 690–693

Sinclair TR (2011) Challenges in breeding for yield increase for drought. Trends Plant Sci. 16:289–293

Smith S, De Smet I (2012) Root system architecture: insights from Arabidopsis and cereal crops. Philos Trans R Soc Lond B Biol Sci. 367:1441-1452

Soller M, Brody T, Genizi A (1976) On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theoretical and Applied Genetics 47:35-39

Steele KA, Virk DS, Kumar R, Prasad SC, Witcombe JR (2007) Field evaluation of upland rice

lines selected for QTLs controlling root traits. Field Crops Res 101:180–186

Sullivan CY (1972) Mechanism of heat and drought resistance in grain sorghum and methods of measurement. In: Rao NGP, House LR, editors. Sorghum in the seventies. New Delhi, India: Oxford & IBH publ Co.; p. 247–64

Szarejko I, Forster BP (2007) Doubled haploidy and induced mutation. Euphytica 158, 359–370

Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald GK (2011) Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. J Exp Bot 62:2189–2203

Teakle NL, Tyerman SD (2010) Mechanisms of Cl^- transport contributing to salt tolerance. Plant Cell Environ 33:566–589

Tester M, Langridge P (2010) Breeding Technologies to Increase Crop Production in a Changing World. Science 327:818-822

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences of the United States of America 108:20260-20264

Tommasini L, Schnurbusch T, Fossati D, Mascher F, Keller B (2007) Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. Theor Appl Genet 115: 697-708

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011) Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. Plant and Soil, 341(1-2), 75-87

Trenberth KE, Jones PD, Ambenje P, et al. 2007. Observations: surface and atmospheric climate change. In: Solomon S, Qin D, Manning M, et al eds. Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.

Tripathy JN, Zhang J, Robin S, Nguyen HT (2000) QTLs for cell-membrane stability mapped in rice (Oryza sativa L.) under drought stress. Theor. Appl. Genet.100:1197–1202

Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci. 11, 405–412

Tuberosa R, Salvi S, Giuliani S, Sanguineti MC, Bellotti M, Conti S, and Landi P (2007) Genome-wide approaches to investigate and improve maize response to drought. Crop Science 47: S120–S141

Tuberosa R (2013) Phenotyping for drought tolerance of crops in the genomics era. Frontiers in Physiology, 3: 347

Turner NC (1979) Drought resistance and adaptation to water deficits in crop plants. In: Mussell H,

Staples CR, eds. Stress physiology in crop plants. New York: John Wiley & Sons, 343–372

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1102. doi:10.1038/ng.2725

Vallega J, Zitelli G (1973) New high yielding Italian durum wheat varieties. In: Proceedings of the Genetics and Breeding of Durum Wheat, Bari, 14–18 May, pp. 373–400

Van Slageren MW (1994) Wild wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub. and Spach) Eig (Poaceae). Agricultural University, Wageningen & International Center for Agricultural Research in Dry Areas, Aleppo, Syria

Varshney RK, Tuberosa R (2007) Application of linkage disequilibrium and association mapping in crop plants. Genomics Approaches and Platforms, Vol 1: 97-119

Varshney RK, Paulo MJ, Grando S, Eeuwijk FA, Keizer LCP, Guo P, Ceccarelli S, Kilian A, Baum M, Graner A (2012) Genome wide association analyses for drought tolerance related traits in barley (Hordeum vulgare L.) Field Crops Res.126:171–180. doi: 10.1016/j.fcr.2011.10.008

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA Wngerprinting. Nucleic Acids Res 23:4407–4414

Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVS, Rebetzke GJ, Kirkegaard JA, Christopher J, Watt M (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63:3485–3498. doi:10.1093/jxb/ers111

Weber J, May P (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Weber A, Clark RM, Vaughn L, Sánchez-Gonzalez JdJ, Yu J, Yandell BS, Bradbury P, Doebley J (2007) Major Regulatory Genes in Maize Contribute to Standing Variation in Teosinte (Zea mays ssp. parviglumis). Genetics 177: 2349-2359

Whittaker JC, Curnow RN, Haley CS, Thompson R (1995) Using marker-maps in marker-assisted selection. Genetical Research, 66: 225-232

Williams J, Kubelik A, Livak K, Rafalski J, Tingey S (1990) DNA polymorphisms ampliWed by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wilson LM, Whitt SR, Rocheford TR, Goodman MM, Buckler ES (2004) Dissection of maize kernel composition and starch production by candidate gene association. Plant Cell 16:2719-2733

Wojciechowski T, Gooding MJ, Ramsay L, Gregory PJ (2009) The effects of dwarfing genes on seedling root growth of wheat. Journal of Experimental Botany 60, 2565–2573

Wu Y, Liu W, Li X, Li M, Zhang D, Hao Z, Weng J, Xu Y, Bai L, Zhang S, Xie C (2011) Lownitrogen stress tolerance and nitrogen agronomic efficiency among maize inbreds: comparison of multiple indices and evaluation of genetic variation. Euphytica 178 (online first)

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Science, 48: 391-407

Yan W, Kang MS (2003) 'GGEBiplot analysis:Agraphical tool for breeders, geneticists, and agronomists.' (CRC Press: Boca Raton, FL)

Yildirim M, Bahar B, Koç M, Barutçular C (2009) Membrane thermal stability at different developmental stages of spring wheat genotypes and their diallel cross populations. Tarim Bilimleri Dergisi, 15 (4): 293-300

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479-501

Yu G-R, Zhuang J, Nakayama K, Jin Y (2007) Root water uptake and profile soil water as affected by vertical root distribution. Plant Ecol 189:15–30. doi:10.1007/s11258-006-9163-y

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457-1468

Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, Tang C, Toomajian C, Zheng H, Dean C, Marjoram P, Nordborg M (2007) An Arabidopsis example of association mapping in structured samples. PLoS Genet 3:e4

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and Prospects of Association Mapping in Plants. 103835/plantgenome2008020089 1:5-20

FIGURES

Fig. 1 Spikes of durum wheat

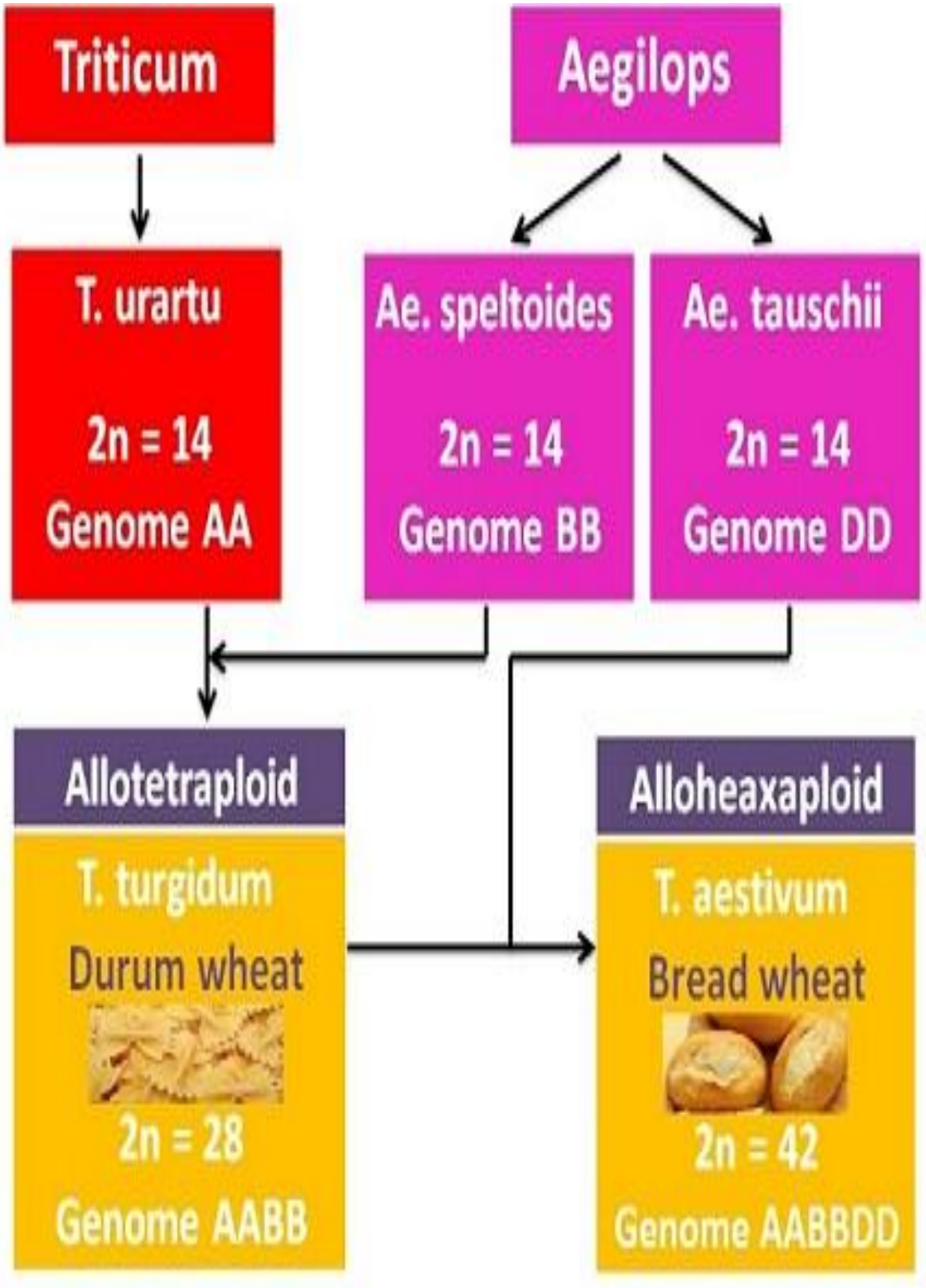

Fig. 2 Durum and Bread wheat evolution

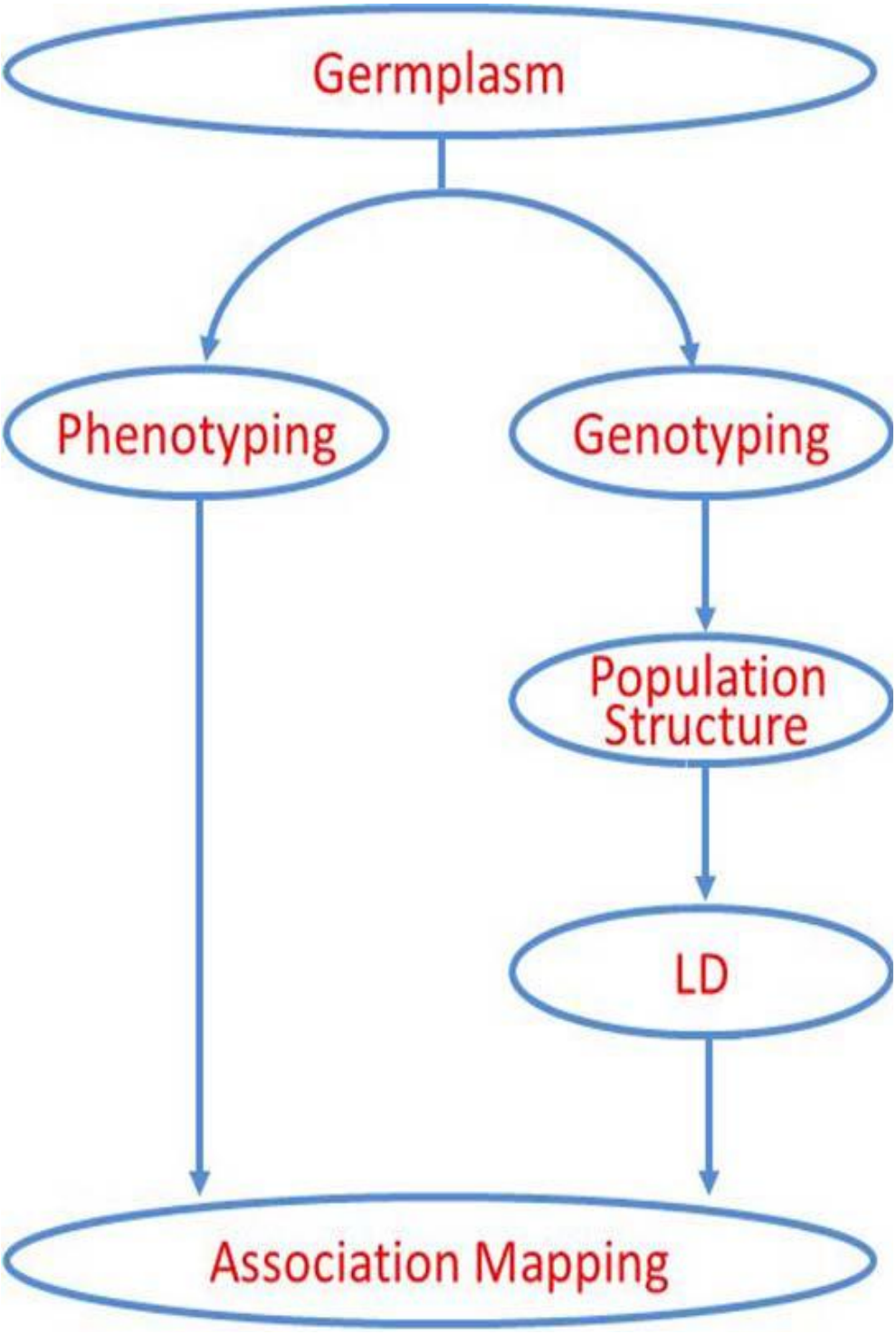

Fig. 3 A simplified flow chart showing different stages of association mapping for tagging a gene of interest using germplasm accessions.

CHAPTER 2. RESEARCH AIMS

The research presented in this thesis aims to identify traits and genes that underlie root system architecture and tolerance to heat stress in durum wheat.

Hence, the objectives of this study were:

- A set of elite durum wheat accessions previously tested for yield and other agronomic traits in 15 field trials carried out by Maccaferri et al. (2011) across a broad range of Mediterranean environments was evaluated at an early growth stage in order to map root system architecture QTLs and verify their effects on grain yield and other agronomic traits.
- Root system architecture traits were evaluated on a recombinant inbred line population from the cross between the cvs. Meridiano and Claudio as a focus for studies the genetic regions controlling and identify QTL associated with root trait architecture in durum wheat.

- Genetic analysis of the variation observed in various anatomical root traits of the durum wheat cultivars is described. To our best knowledge, this is the first investigation of this type in durum wheat. The presence of genetic variation for root anatomical traits and the possibility to rapidly phenotype them could provide novel opportunities for durum wheat breeding programs.

- Association mapping study for heat tolerance in durum wheat was investigated using cell membrane stability (CMS) in a panel of durum wheat accessions well-suited for association mapping studies to detect QTLs associated with heat response traits.

CHAPTER 3. ASSOCIATION MAPPING FOR ROOT ARCHITECTURAL TRAITS IN DURUM WHEAT SEEDLINGS AS RELATED TO AGRONOMIC PERFORMANCE

3.1 ABSTRACT

Association mapping provides useful insights on the genetic architecture of quantitative traits across a large number of unrelated genotypes, which in turn allows an informed choice of the lines to be crossed for a more accurate characterization of major QTLs in a biparental genetic background. In this study, seedlings of 183 durum wheat elite accessions were evaluated in order to identify QTLs for root system architecture (RSA). The QTLs identified were compared with QTLs detected for grain yield and its component traits, plant height and peduncle length measured in a previous study where the same accessions were evaluated in 15 field trials with a broad range of soil moisture availability and productivity (Maccaferri et al. in J Exp Bot 62:409–438, 2011). The following RSA features were investigated in seedlings at the four-leaf stage: seminal root angle, primary root length, total root length, average root length, root number and shoot length. Highly significant differences among accessions were detected for all traits. The highest repeatability (h^2 = 0.72) was observed for seminal root angle. Out of the 48 QTLs detected for RSA, 15 overlapped with QTLs for agronomic traits and/or grain yield in two or more environments. The congruency of the effects of RSA traits and agronomic traits was evaluated. Seminal root angle and root number appear the most promising traits for further studies on the adaptive role of RSA plasticity on field performance in environments differing for water availability. Our results provide novel insights on the genetic control of RSA and its implications on field performance of durum wheat.

Keywords Association mapping, Triticum turgidum L. subsp. durum (Desf.) Husn., QTL, Root architecture, Yield, Drought, Agronomic performance, Root number, Seminal root angle

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3.2 ABBREVIATIONS

AM	Association mapping
ARL	Average root length
DArT	Diversity Array Technology$^{(R)}$ markers
GME_S	General mean of environments
GY	Grain yield
HYE	High-yielding environments
HYE_S^M	Mean of high-yielding environments
KPSM	Number of kernels per square meter
LD	Linkage disequilibrium
LYE	Low-yielding environment
LYE_S^M	Mean of low-yielding environments
MAS	Marker-assisted selection
MYE	Medium-yielding environment
MYE_SM	Mean of medium-yielding environments
PdL	Ear peduncle length
PH	Plant height
PRL	Primary root length
QTL	Quantitative trait locus
RIL	Recombinant inbred line
RSA	Root system architecture
SL	Shoot length
SRA	Seminal root angle
SSR	Simple sequence repeat markers
TKW	Thousand kernel weight
TRL	Total root length
TRN	Total number of roots
TW	Test weight (grain volume weight)

3.3 INTRODUCTION

The fast rise in global food demand coupled with the increasing unpredictability of weather conditions consequent to climate change require the release of cultivars with higher yield potential and able to maintain acceptable yield levels and quality under a broad range of environmental conditions. In view of the quantitative nature of the traits governing yield and yield stability, effectively meeting this formidable challenge will require a multidisciplinary approach based upon both conventional and genomics-assisted breeding practices. Accordingly, major efforts are underway to identify loci (genes and QTLs) for morpho-physiological traits that control yield potential and yield stability, particularly in cereal crops grown across regions characterized by a broad range of water availability (Tuberosa et al. 2007; Fleury et al. 2010; Uga et al. 2013). An example is provided by the Mediterranean Basin, where durum wheat (Triticum durum Desf.) is grown in a range of conditions varying from favorable environments to dryland areas characterized by frequent drought episodes and high temperature stresses, mainly during grain filling (Loss and Siddique 1994; Royo et al. 2010; Maccaferri et al. 2011). Under such conditions, the evaluation of a suitable set of genotypes provides valuable leads for the identification of drought-adaptive traits (Blum 1988; Grando and Ceccarelli 1995; Passioura 2002; Richards 2006; Araus et al. 2008; Reynolds and Tuberosa 2008; Passioura and Angus 2010; Royo et al. 2010; Tardieu and Tuberosa 2010) and the underlying QTLs (Sanguineti et al. 2007; Mathews et al. 2008; Maccaferri et al. 2011; Bennett et al. 2012; Bai et al. 2013; Graziani et al. 2014).

In this context, the study of root architectural system (RSA) features/QTLs as related to crop performance can help to identify proxy traits for enhancing adaptation to different soil properties, moisture conditions, nutrient concentration, etc. (Bacon et al. 2003; Yu et al. 2007; Hochholdinger and Tuberosa 2009; Obara et al. 2010; Sharma et al. 2011; Tuberosa 2012; Lynch 2013; Uga et al. 2013). For example, deep roots might provide a higher protection against dehydration by extracting water stored in deep soil horizons (Ehdaie et al. 2003; Manschadi et al. 2006, 2010; Lilley and Kirkegaard 2007; Hammer et al. 2009; Wasson et al. 2012; Uga et al. 2013). Therefore, identifying and introgressing alleles for deeper rooting in shallowrooted, drought-susceptible cultivars (Grando and Ceccarelli 1995; Steele et al. 2007, 2008; Ehdaie et al. 2010; Uga et al. 2013) is a desirable approach, as underlined by the ‘steep, cheap and deep’ ideotype recently proposed by Lynch (2013).

The evaluation of RSA features directly in the field is very difficult, expensive and time-consuming, especially when dealing with the large number of plants and genotypes required for QTL analysis, particularly with target traits of low heritability (Richards 2008; Christopher et al. 2013). Moreover,

field screening is usually destructive and leads to a substantial loss of the geometry of the root (Nagel et al. 2009). In this respect, it has been reported that adult geometry of the root is strongly related to seminal root angle (SRA), with deeply rooted wheat genotypes showing a narrower SRA, while genotypes with a shallower root system show wider SRA (Manschadi et al. 2008).

Different systems have been adopted to enable an early screening of RSA traits in wheat (Kubo et al. 2007; Sanguineti et al. 2007; Nagel et al. 2009; Munns et al. 2010; Ren et al. 2012; Bai et al. 2013; Christopher et al. 2013; Liu et al. 2013; Watt et al. 2013). In these cases, the assumption is that genotypes that differ in RSA at an early stage would also differ in the field at stages when nutrient and/or water capture is most critical for grain yield.

Among the possible approaches for the functional dissection of quantitative traits, association mapping (AM) has been developed as an alternative to traditional bi-parental linkage mapping to identify associations between phenotypic values of target traits and molecular markers (Ersoz et al. 2007; Sorrells and Yu 2009). In this study, the set of elite durum wheat accessions previously tested for yield and other agronomic traits in 15 field trials carried out by Maccaferri et al. (2011) across a broad range of Mediterranean environments was evaluated at an early growth stage in order to map RSA–QTLs and verify their effects on grain yield and other agronomic traits.

3.4 MATERIALS AND METHODS

3.4.1 Plant material

The panel of 183 elite accessions of durum wheat included cultivars and breeding lines selected in Mediterranean countries (Italy, Morocco, Spain, Syria and Tunisia), Southwestern USA and Mexico that were released from the early 1970s up to the late 1990s (Appendix 1). The panel included also 'founder genotypes' used as parents in breeding programs throughout the Mediterranean Basin and at International CGIAR Centers (CIMMYT and ICARDA). The accessions were chosen according to their pedigree and highly related accessions were excluded. Accessions showing large differences in heading date were excluded to limit possible bias of phenology in the interpretation of the results pertaining to the agronomic traits. A detailed phenotypic and molecular characterization of the panel was previously reported in Maccaferri et al. (2006, 2010, 2011).

3.4.2 Root morphology evaluation

Root morphology was evaluated according to the protocol first described by Bengough et al. (2004), then modified by Sanguineti et al. (2007) and further modified in the present work. For each genotype, 15 seeds were weighed, then sterilized in a 1 % sodium hypochlorite solution for 10 min,

rinsed thoroughly in distilled water and placed in Petri dishes at 28 °C for 24 h. Then, eight homogeneous seedlings with normal seminal root emission were positioned spaced 5 cm from each other on a filter paper sheet placed on a vertical black rectangular (42.5 × 38.5 cm) polycarbonate plate for root obscuration. Distilled water was used for plantlets' growth. Each experimental unit included six plantlets, since the two external ones were considered as border plantlets and, as such, discarded. RSA traits were measured in plantlets that were grown for 9 days at 22 °C under a 16-h light photoperiod. The experiment was conducted according to a randomized complete block design, with three replications in time.

The following traits were investigated on a single plant basis: spread of seminal root angle (SRA), first measured at 3.5 cm from the tip of the seeds as the distance between the two external roots of each plantlet and then converted to degrees, primary root length (PRL), total root length (TRL), total number of roots (TRN), average root length (ARL), and shoot length (SL).

Due to the high number of genotypes under evaluation, the accessions were divided into sets of 25–30 accessions each hereafter reported as blocks. In order to account for possible differences in growth rate among blocks, blocking was taken into account in the subsequent analysis of variance (ANOVA) and a linear adjustment for block effect was carried out. Cultivar Meridiano was also repeated as internal check in every block.

RSA traits were measured on plantlets' images using the software SmartRoot® (Lobet et al. 2011) for all the traits, except for SRA and SL that were measured manually.

3.4.3 Field data

Details and results of the agronomic performance of the panel of accessions were reported in Maccaferri et al. (2011). Briefly, the 183 accessions were tested in 15 field trials carried out during two growing seasons (2003/2004 and 2004/2005) in six countries (Italy, Lebanon, Morocco, Spain, Syria and Tunisia) and in some cases at two water regimes (rainfed and irrigated). Each trial has been coded according to the country (first three letters of each code), the water regime (with 'r' and 'i' standing for rainfed and irrigated trial, respectively) and the year (with 04 and 05 standing for 2004 and 2005, respectively) in which they were conducted. More in detail, three trials were carried out in Italy (Itl1-r04 in Cadriano, 44° 33' N and 11° 24' E; Itl2-r04 and Itl2-r05 in Cerignola, 41° 28' N and 15° 84' E), four in Lebanon (Lbn-r04, Lbn-i04, Lbn-r05, and Lbn-i05 in Rayack, 33° 51' N and 35° 59' E), two in Morocco (Mrc-r04 and Mrc-i04 in SidiElaydi, 31° 15' N and 7° 30' W), two in Spain (Spn1-r04 in Gimenells, 38° 56' N and 0° 29' E; Spn2-r05 in Granada, 37° 15' N and 3° 46' E), two in Syria (Syr-r05 and Syr-i05 in Tel Hadya, 36° 56' N and 36° 04' E) and two in Tunisia

(Tns-r05 and Tns-i05 in Kef, 36° 14' N and 8° 27' E). Each field trial was characterized for the main environmental conditions, i.e., temperature, water availability and soil moisture. For the present study, a re-analysis of agronomic traits was performed with a new genetic map made available at University of Bologna (Maccaferri et al. 2014). In particular, the analysis focused on grain yield (GY), thousand kernel weight (TKW), number of grains per square meter (KPSM), grain volume weight or test weight (TW), plant height (PH) and ear peduncle length (PdL). Based on the GY values reported in Maccaferri et al. (2011), each trial was classified according to its yield level as follows: low-yielding environment (LYE) ranging from 0.9 to 3.6 t ha^{-1}; medium-yielding environment (MYE) ranging from 4.1 to 5.7 t ha^{-1}, high-yielding environment (HYE) ranging from 5.8 to 6.8 t ha^{-1}. Each class included five environments, except LYEs for PH, PdL and TW where only three environments were considered. For each agronomic trait, single environment values and the general mean over all the tested environments (GMEs) were analyzed. The mean values of each environmental class were also included in the analysis (indicated as LYEsM, MYEsM and HYEsM).

On average, in the field trials considered herein the lines of this AM panel showed a heading window of of seven days, with the 70% of the lines heading within two days and 80% within three days (Maccaferri et al. 2011).

3.4.4 Molecular profiling

In the present study, the SSR-based map (334 SSRs) reported in Maccaferri et al. (2011) was enriched with DArT marker. In total, 957 markers (334 SSRs and 623 DArT markers) were used for the molecular profiling of the 183 accessions.

DArT markers were generated by Triticarte Pty Ltd. (Canberra, Australia; http://www.triticarte.com.au). The durum wheat PstI/TaqI array v 2.0, containing 7,600 single DArT clones obtained as described in Mantovani et al. (2008), was used for genotyping the panel. The locus designation used by Triticarte Pty. Ltd. was adopted (‘wPt’, ‘rPt’ and ‘tPt’ loci corresponding to wheat, rye and triticale clones, respectively), and alleles at polymorphic loci were scored as hybridization positive (1) or negative (0).

Markers were ordered according to a consensus map developed at the University of Bologna in the framework of an international cooperation for that purpose (Maccaferri et al. 2014a). Four mapping populations, i.e., Kofa × Svevo (KS RIL population, Maccaferri et al. 2008), Colosseo × Lloyd (CL RIL, Mantovani et al. 2008), Meridiano × Claudio (MC RIL, Maccaferri et al. 2011) and Simeto × Levante (SL RIL, Maccaferri et al. unpublished) were developed by the University of Bologna in

collaboration with Produttori Sementi Bologna SpA (Argelato, BO, Italy). Ten additional maps provided by international partners were used to assemble a common consensus map, used to order the markers available for genotyping the experimental materials herein presented (Maccaferri et al. 2014a).

3.4.5 Statistical analysis and association mapping analysis

The analysis of variance (ANOVA) was conducted on RSA traits based on the mean values of the experimental units. In order to reduce the effect due to blocks, the general mean of each set of genotypes included in the same block was used to correct the corresponding single values, using a linear regression method. To detect possible maternal effects due to seed size, an analysis of covariance was carried out for each trait using kernel weight as covariate.

Repeatability (h^2) was calculated on a mean basis across three replications. Accession means were used to calculate Pearson's correlation coefficients of RSA traits versus the agronomic traits (GY, TKW, KPSM, PH and PdL) for each environment, as well as versus the mean values of each environmental class and the general mean.

To reduce the risk of false-positive marker-trait associations, rare alleles (i.e., with frequencies equal or<0.10) were considered asmissing data. Additionally, marker points showing residual allelic heterogeneity within accession were also considered as missing data; thus, a total of 957 informative markers (i.e., 334 SSR and 623 DArT markers) that was possible to project on the consensus linkage map were utilized for Association Mapping (AM) analysis.

Presence of significant population structure in the panel had been previously shown by Maccaferri et al. (2011) with a combination of model- and distance based analyses using the program STRUCTURE v. 2 (Pritchard et al. 2000). The optimal population structure model was identified by five hypothetical subgroups that led to the Q matrix of membership coefficients of each accession to all subgroups (for details see Maccaferri et al. 2011). Prior to proceeding with AM analysis, a multiple regression analysis was performed to test the significance of the differences among subgroups for the measured RSA traits.

A co-ancestry kinship (*K*) matrix was obtained for the mapped SSR and DArT markers by pairwise genetic similarity values (GS_{ij}) that were calculated for all accession pairs using the simple matching coefficient for multi-state markers. Linkage disequilibrium (LD) was estimated using the program TASSEL, v. 2.1 (www. maizegenetics.net, Yu et al. 2006); *D'* and r^2 values are a function of the corresponding inter-marker distances and the comparison-wise significance was computed with 10,000 permutations. The r^2 LD value was estimated for intra-chromosomal loci and related to

genetic distances between loci (cM). When all pairs of adjacent loci were in LD (r^2 > 0.3), this region was referred to as a LD block (Stich et al. 2005). Genome-wide scans for AM for both RSA traits and agronomic traits were conducted using the TASSEL program, ver. 4.0 (Bradbury et al. 2007). The 334 SSR and 623 DArT markers were tested for significance of marker-trait association under the fixed general linear model (GLM) including the *Q* population structure results as covariates (Q GLM), and the mixed linear model (MLM) including the *Q* population structure results plus the *K* kinship matrix (*Q*+*K* MLM).

For GLM analysis, besides the marker-wise association probability values, the experiment-wise association significance probability was obtained based on a permutation test implemented in TASSEL (10,000 permutations in total). The experiment-wise test provides a much more stringent threshold for significance as compared to the marker-wise test (Bradbury et al. 2007). Three significance levels of marker-trait association were considered, i.e., marker-wise at P = 0.01 [-log(P) = 2.0] and P = 0.001 [-log10(P) = 3.0] and experiment-wise at P = 0.1 [-log10(P) = 4.0, Bonferroni's correction]. The QTL analysis was conducted on both RSA and agronomic traits.

In the present work, only RSA–QTLs co-locating with agronomic traits in at least two environments and/ or on mean values (general means or at least one environmental class mean) are reported. Multiple, adjacent co-segregating significant markers were assigned to a unique QTL region if the strongest marker for the agronomic trait was within 2.5 cM from the reference marker (i.e., where the LOD value was highest) for RSA-QTLs, verifying a significant and strong LD among markers (possibly with r2 values C0.6) (Massman et al. 2011). To facilitate the comparison of the effect of the same chromosomal region on different traits and assess their possible relationship, the effect of each single QTL was always referred to the reference allele (i.e., the allele with the highest frequency) as compared to the overall phenotypic mean at the RSA–QTL peak marker. The allele effect was also reported as percentage of the trait phenotypic mean.

3.5 RESULTS

3.5.1 Phenotypic variation of the accessions' panel for RSA traits

Frequency distributions for RSA traits are shown in Fig. 1, together with the standard deviation estimated on the check cultivar Meridiano, and the LSD based on the ANOVA results. All traits show an approximately normal distribution, indicating a polygenic control. Kernel weight of the samples was taken into account as a covariate in the statistical analysis; the covariate was highly significant for SRA, ARL and TRL, while it was not significant for PRL, TRN and SL. The effect of the significant covariate was taken in due account in the calculation of the adjusted means of the

corresponding traits. No significant regression (data not shown) between phenotypic values of RSA traits and population structure was detected, indicating that the variation observed herein was not influenced by the coefficient of membership of the tested material to the five germplasm subgroups. The experimental material showed a wide range of variation for RSA traits as reported in Table 1 together with the results of the ANOVA. In detail, the RSA traits ranged as follows: SRA from 48° to 147° with a mean value of 100°, PRL from 13.8 to 32.9 cm with a mean value of 21.1 cm, TRL from 52.8 to 144.7 cm with a mean value of 94.6 cm, SL from 7.2 to 16.3 cm with a mean value of 9.7 cm and ARL from 12.0 to 26.0 cm with a mean value of 18.2 cm. TRN showed the lowest variation, from 4.01 to 6.46 roots per plant, with a mean value of 5.18. The ANOVA showed highly significant differences between the genotypes for all traits, with CV values ranging from 6.1% for TRN to 17.0% for PRL. Repeatability values ranged from 48.6% for PRL to 72.8% for SRA. In this respect, it should be underlined that these values are somehow overestimated, due to the fact that the genetic variance includes also the genotype by environment interaction.

3.5.2 Correlation among RSA features and agronomic traits

The analysis of the correlations between RSA traits and agronomic performances of the 183 accessions is reported in Table 2. Correlation coefficients are reported for the mean values of each one of the three environmental classes, namely $LYEs^{M}$, $MYEs^{M}$ and $HYEs^{M}$ as well as for the general mean over all environments (GMEs); additionally, the number of environments showing significant correlations within each class is reported in brackets. Highly significant albei low correlations were detected between SRA and TKW (r = -0.23, -0.21 and -0.20 in $MYEs^{M}$, $HYEs^{M}$ and GMEs, respectively). Accordingly, highly significant and equally low correlations were detected between SRA and TW (r = -0.20, -0.26 and -0.22 in $LYEs^{M}$, $MYEs^{M}$ and GMEs, respectively). Moreover, SRA showed significant correlations with PdL in $LYEs^{M}$ (r = -0.20), $HYEs^{M}$ (r = -0.19) and GMEs (r = -0.20). All these correlations showed a significant, albeit low, negative value, thus suggesting that a more superficial root system (i.e., increase in SRA) is associated with a decreased PdL, TKW and TW. SRA was also significantly correlated with KPSM in $MYEs^{M}$ (r = 0.23), $HYEs^{M}$ (r = 0.24) and GMEs (r = 0.23). A significant, positive correlation was observed between SL and PH in $MYEs^{M}$ (r = 0.19), $HYEs^{M}$ (r = 0.21) and GMEs (r = 0.20).
As to GY, significant albeit low correlations were only detected with TRN in $LYEs^{M}$ and GMEs (r = 0.24 and 0.18, respectively). No additional significant correlation was detected between GY and other RSA traits.

3.5.3 QTL analysis for RSA features and agronomic traits

The results of AM analysis are reported in Table 3 and in Supplementary Table 1. QTLs are reported ordered according to their map position. In total, we identified 10 QTLs for SRA, 11 for PRL, 10 for ARL, 8 for TRL, 4 for TRN and 5 for SL. Among these 48 QTLs, 15 overlapped with QTLs for agronomic traits. Among these 15 QTLs, three (i.e., *QARL$_1$-2A*, *QSRA$_4$-6A* and *QSRA$_6$-6B*) were significant at marker-wise significance level of $P < 0.001$ (-log10 > 3.0), while the other 12 were significant at the marker-wise significance level of $P < 0.01$ (-log10 > 2.0); none of these QTLs exceeded the experiment-wise threshold computed based on the Bonferroni's correction, a highly conservative approach as to Type I error. The QTLs described hereafter are identified according to the RSA traits for which the QTLs were detected; in case the same QTL affected more than one RSA trait, the QTL is named after the trait showing the highest *P* value. The overlap with QTLs for GY, TW, TKW, KPSM, PH and PdL is also reported.

For the sake of clarity, we wish to point out that whenever the relative effects of RSA-QTL alleles on root traits were positively or negatively associated to the effects on grain yield and other agronomic traits, these concurrent effects are defined as "congruent" and "contrasting", respectively.

3.5.4 QTLs for seminal root angle

Among the 15 QTLs that overlapped with SRA-QTLs, six were identified for SRA on chromosomes 1B, 3A, 4B, 6A and 6B, with R^2 values ranging from 4.59% (*QSRA$_5$-6B*) to 7.74% (*QSRA$_4$-6A*). None of these QTLs for SRA co-located with QTLs for other RSA features measured in this study. Among these six SRA-QTLs, three (*QSRA$_3$-4B*, *QSRA$_5$-6B* and *QSRA$_6$-6B*) co-located with GY-QTLs in at least two environments, while *QSRA$_1$-1B* co-located with GY-QTLs in one environment only. *QSRA$_3$-4B* co-located with GY-QTLs in three environments (two LYEs and one MYE), in LYE^M, MYEs^M and GMEs; notably, the effects estimated for GY were congruent with those estimated for SRA. *QSRA$_5$-6B* co-located with GY in two HYEs; the effects estimated for GY congruent with those estimated fof SRA. *QSRA$_6$-6B* co-located with GY in two environments (one LYE and one HYE) and in HYEs^M; in this case, the allelic effects for SRA and GY were congruent in one HYE and HYEs^M but contrasting in the LYE.

Considering GY components (i.e., TKW and KPSM), four out of the six SRA-QTLs (all except *QSRA$_1$-1B* and *QSRA$_2$-3A*) co-located with QTLs for at least one of these traits in two or more environments. In detail, *QSRA$_3$-4B* co-located with KPSM in one MYE (with contrasting effects in comparison with SRA) and in LYEs^M, with an affect congruent with SRA; moreover, the same

QTL co-located also with TKW in one HYE, with an effect in contrast with SRA. *QSRA$_4$-6A* co-located with TKW in five environments (one LYE, two MYEs and two HYEs) as well as with TKW in LYEs^M and HYEs, all showing effects congruent with those on TKW and SRA. *QSRA$_5$-6B* co-located with TKW in LYEs^M with a consistent effect for TKW and SRA; even though this QTL overlapped with LYEs^M only, it is noteworthy because its effects on the mean of five environments suggests a more prominent role for this QTL. *QSRA$_6$-6B* overlapped with QTLs for KPSM in one LYE and in one MYE, both of which showed contrasting effects as compared to SRA.

Considering the other agronomic traits, two RSA-QTLs appear particularly interesting for their co-location with QTLs for TW, a trait closely related to grain quality and to starch accumulation capacity in the final phase of grain filling in durum wheat. *QSRA$_1$-1B* co-located with TW-QTLs in four environments (one LYE, one MYE and two HYEs), all with contrasting effects as compared to SRA except for one HYE (Itl1-r04), where consistent effects were noted, Additionally, this QTL influenced GY in one LYE, with contrasting effects as compared to SRA. Moreover, *QSRA$_2$-3A* influenced TW in three environments, two MYEs (with effects congruent with SRA) and one HYE (with an effect contrasting with that on SRA). *QSRA$_4$-6A* influenced TW in one LYE and in LYEs^M, with contrasting effects with those on SRA in both cases. In two MYEs, TW was influenced also by *QSRA$_5$-6B*, in both cases with congruent effects on TW.

Finally, two SRA-QTLs co-located with QTLs for morphological traits at the adult stage of the plants in the field. *QSRA$_3$-4B* influenced PH in three environments (two HYEs and one MYE) and in HYEs^M, in all cases with contrasting effects on PH. Moreover, *QSRA$_3$-4B* influenced PdL in the least productive LYE (Spn2-r05), also in this case with an effect in contrast to those on SRA. *QSRA$_6$-6B* showed contrasting effects on PdL in three environments (one LYE, one MYE and one HYE).

3.5.5 QTLs for total root number

Two QTLs for TRN (*QTRN$_1$-3A* and *QTRN$_2$-4B*) overlapped with QTLs for agronomic traits. *QTRN$_1$-3A* (R^2 = 5.10%) co-located with KPSM-QTLs in eight environments (four HYEs, three MYEs and one LYE) as well as in HYEs^M, MYEs^M and in GMEs, in all cases with effects congruent with those on TRN. Moreover, *QTRN$_1$-3A* co-located with TKW-QTLs in five environments (four HYEs and one MYE) and in HYEs^M, in all cases with contrasting effects as compared to those on TRN. *QTRN$_1$-3A* co-located also with TW-QTLs in two environments (one HYE and one MYE with contrasting and congruent effects, respectively) and in HYEs^M (with contrasting effects). Finally, it overlapped with PH-QTLs in six environments (four HYEs and two

MYEs) and with PdL-QTLs in two environments (one MYE and one HYE), in all cases with contrasting effects

QTRN$_2$-4B (R^2 = 5.59%) co-located with TKW-QTLs in two HYEs (with contrasting effects) and one MYE (with congruent effects). *QTRN$_2$-4B* co-located also with KPSM-QTLs in three environments (with contrasting effecst in two MYEs and congruent effects in one HYE). *QTRN$_2$-4B* also co-located with a GY-QTL in one HYE environment, with contrasting effects.

3.5.6 QTLs for primary, total and average root length

Among the six QTLs that were identified, two (*QARL$_1$-2A* and *QPRL$_2$-2B*) co-located for PRL, TRL and ARL (the identification acronym was attributed based on the trait with the highest R^2 value), while the other four QTLs were specific for only one of these RSA traits. *QARL$_1$-2A* was identified on chr. 2A, with R^2 values of 9.41% for ARL, 7.33% for TRL and 5.56% for PRL. In all cases, the reference allele showed negative effects for these RSA traits. *QARL$_1$-2A* influenced KPSM in seven environments (three LYEs, two MYEs and two HYEs) and in GMEs, in all cases with congruent effects. Moreover, *QARL$_1$-2A* co-located with TW-QTLs in two environments (one LYE and one MYE), in MYEs^M and in GMEs, in both cases with contrasting effects. *QPRL$_2$-2B* was detected on chr. 2B, with R^2 values equal to 5.83% for PRL, 5.25% for ARL and 4.21% for TRL. This QTL showed a congruent effect on GY in one LYE and a small contrasting effect in one HYE. It co-located also with TW-QTLs in two HYEs, in MYEs^M and in GMEs, always with congruent effects.

Considering the scored RSA traits, PRL was influenced by *QPRL$_1$-1B* and *QPRL$_3$-4A* with R^2 values of 6.18 and 4.47%, respectively, both showing congruent effects. *QPRL$_1$-1B* co-located with TW-QTLs in LYEs^M (with a consistent effect) as well as in HYEs^M and in GMEs (in both cases with contrasting effects). *QPRL$_3$-4A* co-located with KPSM-QTLs in two environments (one HYE and one MYE, both with consistent effects) and with TKW-QTLs in the same HYE (Itl2-r04) with contrasting effects.

As to TRL, *QTRL$_1$-6B* (R^2 = 5.32%) co-located with a GY-QTL in GMEs (with a consistent effect) and with a TKW-QTL in LYE^M (with a contrasting effect). Moreover, it co-located with KPSM-QTLs in one MYE and in MYEs^M, in both cases with consistent effects. It co-located also with PH-QTLs in three environments as well as in MYEs^M, HYEs^M and GMEs, and with PdL-QTLs in four environments, HYEs^M and GMEs. At this QTL, the reference allele negatively affected both PH and PdL while affecting positively TRL.

As to ARL, *QARL$_2$-7B* (R^2 = 4.67%) co-located with TKW in four environments (one LYE, two MYEs and one HYE), with contrasting effects; moreover, *QARL$_2$-7B* co-located with TW in two environments (one LYE with a consistent effect and one MYE with a contrasting effect). It co-located also with KPSM-QTLs in one HYE, in MYEs^M and in GMEs, in all cases with effects consistent with those on ARL. Finally, it co-located with one PdL-QTL in LYEs^M, showing a contrasting effect.

3.5.7 QTLs for shoot length

Only one QTL identified for SL co-located with agronomic traits. *QSL1-3A* (R^2 = 4.14%) co-located with TW-QTLs in one HYE and in GMEs, showing consistent positive effects in both cases, but contrasting with SL effect. Additionally, *QSL1-3A* co-located with QTLs for KPSM and TKW in one HYE, with a contrasting effect on SL, and a congruent one on TKW.

3.6 DISCUSSION

A valuable feature of the panel of genotypes evaluated in this study is their limited range in heading time as previously reported (Maccaferri et al. 2011). Limited variability in phenology is of utmost importance for a meaningful interpretation of studies to investigate the role of drought-adaptive features on field performance across environments characterized by large variability in soil moisture during the reproductive stage, a factor that plays a key role in setting yield potential particularly in Mediterranean environments (Araus et al. 2003a, b; Garcia del Moral et al. 2003; Royo et al. 2010).

3.6.1 Phenotypic variation for RSA traits

A number of approaches/techniques have been developed for the description of RSA in controlled environments at different levels of throughput and cost (Tuberosa et al. 2002; Sanguineti et al. 2007; Nagel et al. 2009; Zhu et al. 2011; Grossman and Rice 2012; Pacheco Villalobos and Hardtke 2012; Postma and Lynch 2012; Bai et al. 2013; Lavenus et al. 2013; Watt et al. 2013; Wasson et al. 2014). The approach utilized herein allows for a reasonably rapid and accurate phenotyping of RSA in hundreds of plants, as usually required by any QTL study.

With the exception of TRN, the durum accessions tested herein have shown a range of variation (from two up to three fold in magnitude) and repeatability (from 48.6 % for PRL to 72.8 % for SRA) for RSA traits that appears suitable for further investigation. These results are particularly noteworthy considering that the tested materials are mainly elite cultivars that usually explore only a limited portion of the variability present in the genepool available for each species. The variability

found for RSA features may to a certain extent reflect the adaptive value of such features for the environmental conditions prevailing in the original selection sites of each cultivar. Therefore, this experimental material provides further opportunities for dissecting RSA complexity and its possible functional role in field performance and grain yield plasticity of durum wheat.

3.6.2 Correlation among RSA features and agronomic traits

Overall, the correlations between RSA features and agronomic traits were very low, not at all unexpectedly in consideration that RSA data were measured at a very early stage and in growing conditions unable to properly mimic soil conditions, hence unable to account for RSA plasticity and its adaptive role for grain yield (GY) in the field. This notwithstanding, once the variability of phenotypic values was dissected at the QTL level, the analysis of RSA data and agronomic performance has revealed several concurrent QTL effects on RSA, GY and other agronomic traits. Other studies conducted in maize (Landi et al. 2007, 2010) and rice (Steele et al. 2007; Uga et al. 2013) grown under controlled conditions have revealed sizeable, concurrent effects of QTLs for RSA features on GY and other agronomic traits evaluated under field conditions, thus providing valuable opportunities for genomics-assisted breeding approaches, like in the case of rice (Steele et al. 2006).

Among the investigated root traits, SRA was negatively correlated with TKW and TW, a result possibly due to the influence of root angle on root distribution in soil layers, hence on water uptake from deeper soil horizons (Manschadi et al. 2010; Lynch 2013; Lynch et al. 2014). SRA was also correlated with both KPSM (positive association) and TKW (negative association) in $MYEs^{M}$, $HYEs^{M}$ and GMEs. These findings account for the lack of association of SRA with GY since a counterbalancing effect between the two main yield components inevitably leads to a lack of significant effects of such variability on GY itself.

The positive, albeit low, correlation observed between TRN and GY in $LYEs^{M}$ and also GMEs suggests a beneficial adaptive role of TRN on GY in environments with low yield potential due to unfavorable growth conditions, consistently with the study conducted by Liu et al. (2013) on RSA traits and GY in wheat at two different water regimes. Notably, among the RSA features herein investigated TRN was the trait with the highest correlation with GY. These results could be ascribed to the fact that a higher number of seminal roots provide greater early vigor a trait known to be particularly crucial for enhancing water uptake in drought-prone environments (Blum 1996; Richards 2006, 2008; Reynolds and Tuberosa 2008). It is noteworthy that in the study conducted by Liu et al. (2013), focusing on RSA traits and GY at two different water regimes, TRN was the trait

with the highest correlation with GY. Accordingly, we observed a positive correlation between SL and PH in $MYEs^{M}$, $HYEs^{M}$ and GMEs, a result that further underlines the importance of early seedling growth on yield performance of wheat.

3.6.3 QTL analysis for RSA features and agronomic traits

The large number of QTLs (48 in total) for RSA features evidenced in our study underlines the complexity of the genetic control of these traits already at an early growth stage. Previous QTL studies conducted on the same set of genotypes considered herein have revealed striking differences as to the role of specific QTLs on specific traits when the genetic dissection was based upon biparental mapping (Maccaferri et al. 2008; Graziani et al. 2014) and association mapping (Maccaferri et al. 2011). Therefore, a more exhaustive search for novel haplotypes governing RSA traits in durum wheat should deploy larger and more genetically diverse panels as well as biparental mapping populations, preferably derived from non-elite materials such as landraces and wild relatives (e.g., emmer wheat and T. dicoccoides) more likely to carry novel alleles for RSA features conferring adaptation to water-limited conditions. The use of high density SNP maps (Trebbi et al. 2011; Van Poecke et al. 2013; Maccaferri et al. 2014a, b) coupled with sequencing information will facilitate the identification of novel haplotypes and in some case may also provide valuable clues on the possible candidates underlying root phenotypes. Along this line, the high LD of elite durum wheat germplasm (Maccaferri et al.
2005, 2006) does not allow for meaningful speculation on the possible role of genes syntenic to candidates that have been suggested to control RSA features in other cereals.

Approximately, 30 % (15/48) of the SRA–QTLs concurrently affected agronomic traits including also GY and/or its main components, thus providing circumstantial albeit valuable evidence as to the implications of RSA variability at an early growth stage on the field performance of durum wheat.

The RSA trait with the most extensive overlap with agronomic performance was SRA, a feature of particular interest in both durum and bread wheat as recently highlighted by Christopher et al. (2013) since the angle of roots at their emergence from the seeds could be a valuable proxy for rooting depth (Kato et al. 2006; Wasson et al. 2012). Accordingly, modeling of RSA features suggests that a narrow angle of wheat roots could lead, in general, to deeper root growth and higher yields (de Dorlodot et al. 2007; Manschadi et al. 2008; Wasson et al. 2012; Lynch 2013). In the present study, considering the results obtained for the single QTLs, the relationship between GY, GY component traits and SRA varied according to the level of yield potential of each particular

location, consistently with the findings of Christopher et al. (2013) in bread wheat, thus indicating that the optimal root angle ideotype is likely to vary according to the target environment. Other studies have underlined the specificity of the response of GY to RSA features in different environments. As reported by Wasson et al. (2012), in wheat, the same RSA features led to markedly different GY responses according to the environment in which those materials were first selected and then cultivated (Oyanagi et al. 1993; Manschadi et al. 2008). Therefore, if experimental evidence suggests that SRA in seedlings might be closely related to adult plant rooting depth, the field conditions in which the crop is grown determine the final performance in a given environment (White and Kirkegaard 2010; Wasson et al. 2012). In the present study, the six QTL regions that influenced SRA and agronomic performance showed contrasting relationships as to the effects of SRA on GY and its components. Contrasting effects of a specific drought-adaptive QTL on GY as a function of different environmental conditions have been previously reported and the underlying reasons critically discussed (Collins et al. 2008). In this respect, particularly noteworthy is the case of *$QSRA_6$-6B*, where SRA and GY effects were negatively associated in Spn2-r05, a LYE devoid of moisture in the superficial soil horizon (Maccaferri et al. unpublished) usually more massively explored by root systems with a wider SRA. Conversely, SRA and GY effects at *$QSRA_6$-6B* were positively associated in $HYEs^M$, possibly due to the fact that shallow roots have been shown to more effectively acquire mobile and immobile nutrients that in fertile soils tend to be more abundant in topsoil layers (Lynch 2013). Notably, a PH-QTL has been mapped to the same position in durum wheat (Sanguineti et al. 2007), a finding consistent with the effects of the same region reported in the present work for PdL, the main component of PH in durum wheat (Maccaferri et al. 2008). A similar relationship between SRA with GY and TW was observed for *$QSRA_1$-1B*, where SRA was negatively related to GY in a LYE and to TW in one environment of each one of the three yield classes (LYE, MYE, HYE); however, a positive association with the QTL effects on TW was observed in P3r04, the second highest yielding environment. At the other four SRA-QTLs the effects on SRA and GY-QTL were positively related. Among these four QTLs, *$QSRA_3$-4B* showed a negative association of SRA with PH mainly in HYEs as well as with PdL in Spn2-r05 a LYE.

Interestingly, *$QSRA_3$-4B* co-located with a QTL identified by Ren et al. (2012) for root length-related traits in bread wheat, thus highlighting the importance of this region in governing RSA in both species and making this QTL a valuable candidate for fine mapping and cloning. In our study, also *$QSRA_4$-6A* showed concurrent effects on TKW and SRA, but not on TW in P4r05, the less yielding environment, and $LYEs^M$, suggesting again a positive effect of a potentially deeper root

systems in extremely poor and dry environments. This hypothesis is further supported by the co-location of *QSRA$_4$-6A* with the QTL identified by Kubo et al. (2007) for penetration ability of the root in deeper soil layers, consistently with the root ideotype proposed by Lynch (2013) as a means to allow the plant to more effectively explore deeper soil levels and capture higher amounts of soil moisture.

Among the QTLs detected for RSA traits and overlapping with agronomic features, six were related to root length. In general, at these QTLs a positive association between root length and agronomic performance was observed, mainly in environments with lower water availability.

3.7 CONCLUSIONS

Notwithstanding the critical role played by roots on the agronomic performance of wheat, so far only two studies have addressed the implications of RSA–QTLs of seedlings to field performance in wheat (Sanguineti et al. 2007; Bai et al. 2013). Our study has unveiled the presence of several novel RSA–QTLs while highlighting those with concurrent effects also on agronomic traits and yield under field conditions. Among RSA traits, seminal root angle appears the most promising for undertaking further studies on the role of RSA on field performance. Based upon the results herein reported, we have developed biparental RIL populations obtained from the cross of accessions contrasted for root angle and other RSA features in order to more accurately assess the genetic basis of RSA in durum wheat and the effects of the most relevant RSA–QTL haplotypes on GY in different water regimes. Eventually, this information might lead to the identification of RSA loci worthy of a MAS approach aimed to enhance yield potential and yield stability of durum wheat grown under different soil moisture conditions.

3.8 REFERENCES

Araus JL, Bort J, Steduto P, Villegas D, Royo C (2003a) Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. Ann Appl Biol 142:129–141

Araus JL, Villegas D, Aparicio N, del Moral LFG, El Hani S, Rharrabti Y, Ferrio JP, Royo C (2003b) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci 43:170–180

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Crit Rev Plant Sci 27:377–412. doi:10.1080/07352680802467736

Bacon MA, Davies WJ, Mingo D, Wilkinson S (2003) Root signals. Roots: the hidden half. Marcel Dekker Inc, New York, pp 460–471

Bai C, Liang Y, Hawkesford MJ (2013) Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. J Exp Bot 64:1745–1753. doi:10.1093/jxb/ert041

Bengough AG, Gordon DC, Al-Menaie H, Ellis RP, Allan D, Keith R, Thomas WTB, Forster BP (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant Soil 262:63–70

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012) Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water-limited environments. Theor Appl Genet 125:255–271. doi:10.1007/s00122-012-1831-9

Blum A (1988) Breeding for stress environments. CRC Press Inc., Boca Raton

Blum A (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regul 20:135–148

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635. doi:10.1093/bioinformatics/btm308

Christopher J, Christopher M, Jennings R, Jones S, Fletcher S, Borrell A, Manschadi AM, Jordan D, Mace E, Hammer G (2013) QTL for root angle and number in a population developed from bread wheats (Triticum aestivum) with contrasting adaptation to water-limited environments. Theor Appl Genet 126:1563–1574. doi:10.1007/s00122-013-2074-0

Collins NC, Tardieu F, Tuberosa R (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol 147:469–486. doi:10.1104/pp.108.118117

de Dorlodot S, Forster B, Pages L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci 12:474–481

del Moral LFG, Rharrabti Y, Villegas D, Royo C (2003) Evaluation of grain yield and its components in durum wheat under Mediterranean conditions: an ontogenic approach. Agron J 95:266–274

Ehdaie B, Whitkus RW, Waines JG (2003) Root biomass, wateruse efficiency, and performance of wheat-rye translocations of chromosomes 1 and 2 in spring bread wheat 'Pavon'. Crop Sci 43:710–717

Ehdaie B, Merhaut DJ, Ahmadian S, Hoops AC, Khuong T, Layne AP, Waines JG (2010) Root system size influences water-nutrient uptake and nitrate leaching potential in wheat. J Agron Crop Sci 196:455–466. doi:10.1111/j.1439-037X.2010.00433.x

Ersoz ES, Yu J, Buckler ES (2007) Applications of linkage disequilibrium and association mapping. In: Varshney RK, Tuberosa R (eds) Genomics-assisted crop improvementvol 1: genomics approaches and platforms, vol 1. Springer, New York, pp 97–120

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. J Exp Bot 61:3211–3222. doi:10.1093/jxb/erq152

Grando S, Ceccarelli S (1995) Seminal root morphology and coleoptile length in wild (Hordeum vulgare ssp. spontaneum) and cultivated (Hordeum vulgare ssp. vulgare) barley. Euphytica 86:73–80

Graziani M, Maccaferri M, Royo C, Salvatorelli F, Tuberosa R (2014) QTL dissection of yield components and morphophysiological traits in a durum wheat elite population tested in contrasting thermo-pluviometric conditions. Crop and Pasture Science 65:80–95

Grossman JD, Rice KJ (2012) Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. Evol Appl 5:850–857. doi:10.1111/j.1752-4571.2012.00263.x

Hammer GL, Dong ZS, McLean G, Doherty A, Messina C, Schusler J, Zinselmeier C, Paszkiewicz S, Cooper M (2009) Can changes in canopy and/or root system architecture explain historical maize yield trends in the US Corn Belt? Crop Sci 49:299–312. doi:10.2135/cropsci2008.03.0152

Hochholdinger F, Tuberosa R (2009) Genetic and genomic dissection of maize root development and architecture. Curr Opin Plant Biol 12:172–177. doi:10.1016/j.pbi.2008.12.002

Kato Y, Abe J, Kamoshita A, Yamagishi J (2006) Genotypic variation in root growth angle in rice (Oryza sativa L.) and its association with deep root development in upland fields with different water regimes. Plant Soil 287:117–129. doi:10.1007/s11104-006-9008-4

Kubo K, Elouafi L, Watanabe N, Nachit MM, Inagaki MN, Iwama K, Jitsuyama Y (2007) Quantitative trait loci for soil-penetrating ability of roots in durum wheat. Plant Breed 126:375–

378

Landi P, Sanguineti MC, Liu C, Li Y, Wang TY, Giuliani S, Bellotti M, Salvi S, Tuberosa R (2007) Root-ABA1 QTL affects root lodging, grain yield, and other agronomic traits in maize grown under well-watered and water-stressed conditions. J Exp Bot 58:319–326

Landi P, Giuliani S, Salvi S, Ferri M, Tuberosa R, Sanguineti MC (2010) Characterization of root-yield-1.06, a major constitutive QTL for root and agronomic traits in maize across water regimes. J Exp Bot 61:3553–3562. doi:10.1093/jxb/erq192

Lavenus J, Goh T, Roberts I, Guyomarc'h S, Lucas M, De Smet I, Fukaki H, Beeckman T, Bennett M, Laplaze L (2013) Lateral root development in Arabidopsis: fifty shades of auxin. Trends Plant Sci 18:455–463. doi:10.1016/j.tplants.2013.04.006

Lilley JM, Kirkegaard JA (2007) Seasonal variation in the value of subsoil water to wheat: simulation studies in southern New South Wales. Aust J Agric Res 58:1115–1128. doi:10.1071/ar07046

Liu X, Li R, Chang X, Jing R (2013) Mapping QTLs for seedling root traits in a doubled haploid wheat population under different water regimes. Euphytica 189:51–66. doi:10.1007/s10681-012-0690-4

Lobet G, Pages L, Draye X (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol 157:29–39. doi:10.1104/pp.111.179895

Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Adv Agron 52:229–276. doi:10.1016/s0065-2113(08)60625-2

Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357. doi:10.1093/aob/mcs293

Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. J Exp Bot. doi:10.1093/jxb/eru162

Maccaferri M, Sanguineti MC, Noli E, Tuberosa R (2005) Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Mol Breed 15(3):271–289. doi:10.1007/s11032-004-7012-z

Maccaferri M, Sanguineti MC, Natoli V, Ortega JLA, Salem MB, Bort J, Chenenaoui C, De Ambrogio E, del Moral LG, De Montis A, El-Ahmed A, Maalouf F, Machlab H, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Tuberosa R (2006) A panel of elite accessions of durum wheat (Triticum durum Desf.) suitable for association mapping studies. Plant Genet Resour 4:79–85. doi:10.1079/PGR2006117

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Ben Salem M, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178:489–511. doi:10.1534/genetics.107.077297

Maccaferri M, Sanguineti MC, Mantovani P, Demontis A, Massi A, Ammar K, Kolmer JA, Czembor JH, Ezrati S, Tuberosa R (2010) Association mapping of leaf rust response in durum wheat. Mol Breed 26:189–228. doi:10.1007/s11032-009-9353-0

Maccaferri M, Sanguineti MC, Demontis A, El-Ahmed A, del Moral LG, Maalouf F, Nachit M, Nserallah N, Ouabbou H, Rhouma S, Royo C, Villegas D, Tuberosa R (2011) Association mapping in durum wheat grown across a broad range of water regimes. J Exp Bot 62:409–438. doi:10.1093/jxb/erq287

Maccaferri M, Cane MA, Salvi S, Noli E, Colalongo C,Massi A, Clarke F, Pozniak C, Korol A, Fahima T, Dubcovsky J, Xu S, Karsai I, Knox R, Clarke J, Tuberosa R (2014a) A consensus framework map of durum wheat (Triticum durum Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. BMC Genomics 15:873

Maccaferri M, Ricci A, Salvi S, Milner SG, Noli E, Martelli PL, Casadio R, Akhunov E, Scalabrin S, Vendramin V, Ammar K, Blanco A, Desiderio F, Distelfeld A, Dubcovsky J, Fahima T, Faris J, Korol A, Massi A, Mastrangelo AM, Morgante M, Pozniak C, N'Diaye A, Xu S, Tuberosa R (2014b) A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnol J. doi:10.1111/pbi.12288

Manschadi AM, Christopher J, Devoil P, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. Funct Plant Biol 33:823–837

Manschadi AM, Hammer GL, Christopher JT, deVoil P (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (Triticum aestivum L.). Plant Soil 303:115–129. doi:10.1007/s11104-007-9492-1

Mantovani P, Maccaferri M, Sanguineti MC, Tuberosa R, Catizone I, Wenzl P, Thomson B, Carling J, Huttner E, DeAmbrogio E, Kilian A (2008) An integrated DArT-SSR linkage map of durum wheat. Mol Breed 22: 629–648

Manschadi AM, Christopher JT, Hammer GL, Devoil P (2010) Experimental andmodelling studies of drought-adaptive root architectural traits in wheat (Triticum aestivum L.). Plant Biosyst 144:458–462. doi:10.1080/11263501003731805

Massman J, Cooper B, Horsley R, Neate S, Dill-Macky R, Chao S, Dong Y, Schwarz P, Muehlbauer GJ, Smith KP (2011) Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. Mol Breed 27:439–454. doi:10.1007/s11032-010-9442-0

Mathews KL, Malosetti M, Chapman S, McIntyre L, Reynolds M, Shorter R, van Eeuwijk F (2008) Multi-environment QTL mixed models for drought stress adaptation in wheat. Theor Appl Genet 117:1077–1091. doi:10.1007/s00122-008-0846-8

Munns R, James RA, Sirault XRR, Furbank RT, Jones HG (2010) New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. J Exp Bot 61:3499–3507. doi:10.1093/jxb/erq199

Nagel KA, Kastenholz B, Jahnke S, Van Dusschoten D, Aach T, Muehlich M, Truhn D, Scharr H, Terjung S, Walter A, Schurr U (2009) Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. Funct Plant Biol 36:947–959. doi:10.1071/fp09184

Obara M, Tamura W, Ebitani T, Yano M, Sato T, Yamaya T (2010) Fine-mapping of qRL6.1, a major QTL for root length of rice seedlings grown under a wide range of $NH4^+$ concentrations in hydroponic conditions. Theor Appl Genet 121:535–547. doi:10.1007/s00122-010-1328-3

Oyanagi A, Nakamoto T, WadaM(1993) Relationship between root-growth angle of seedlings and vertical-distribution of roots in the field in wheat cultivars. Jpn J Crop Sci 62:565–570

Pacheco-Villalobos D, Hardtke CS (2012) Natural genetic variation of root system architecture from Arabidopsis to Brachypodium: towards adaptive value. Philos Trans R Soc Biol Sci 367:1552–1558. doi:10.1098/rstb.2011.0237

Passioura JB (2002) Environmental biology and crop improvement. Funct Plant Biol 29:537–546. doi:10.1071/fp02020

Passioura JB, Angus JF (2010) Improving productivity of crops in water-limited environments. Adv Agron 106:37–75. doi:10.1016/s0065-2113(10)06002-5

Postma JA, Lynch JP (2012) Complementarity in root architecture for nutrient uptake in ancient maize/bean and maize/bean/squash polycultures. Ann Bot 110:521–534. doi:10.1093/aob/mcs082

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association mapping in structured populations. Am J Hum Genet 67:170–181

Ren Y, He X, Liu D, Li J, Zhao X, Li B, Tong Y, Zhang A, Li Z (2012) Major quantitative trait loci for seminal root morphology of wheat seedlings. Mol Breed 30:139–148. doi:10.1007/s11032-

011-9605-7

Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. Curr Opin Plant Biol 11:171–179. doi:10.1016/j. pbi.2008.02.005

Richards RA (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. Agric Water Manag 80:197–211. doi:10.1016/j.agwat.2005.07.013

Richards RA (2008) Genetic opportunities to improve cereal root systems for dryland agriculture. Plant Prod Sci 11:12–16

Royo C, Maccaferri M, Alvaro F, Moragues M, Sanguineti MC, Tuberosa R, Maalouf F, del Moral LFG, Demontis A, Rhouma S, Nachit M, Nserallah N, Villegas D (2010) Understanding the relationships between genetic and phenotypic structures of a collection of elite durum wheat accessions. Field Crops Res 119:91–105. doi:10.1016/j.fcr. 2010.06.020

Sanguineti MC, Li S, Maccaferri M, Corneti S, Rotondo F, Chiari T, Tuberosa R (2007) Genetic dissection of seminal root architecture in elite durum wheat germplasm. Ann Appl Biol 151:291–305. doi:10.1111/j.1744-7348.2007.00198.x

Sharma S, Xu S, Ehdaie B, Hoops A, Close TJ, Lukaszewski AJ, Waines JG (2011) Dissection of QTL effects for root traits using a chromosome arm-specific mapping population in bread wheat. Theor Appl Genet 122:759–769. doi:10.1007/s00122-010-1484-5

Sorrells ME, Yu J (2009) Linkage disequilibrium and association mapping in the Triticeae. Genet Genomics Triticeae 7:655–683. doi:10.1007/978-0-387-77489-3_22

Steele KA, Price AH, Shashidhar HE, Witcombe JR (2006) Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theor Appl Genet 112:208–221

Steele KA, Virk DS, Kumar R, Prasad SC, Witcombe JR (2007) Field evaluation of upland rice lines selected for QTLs controlling root traits. Field Crops Res 101:180–186

Stich B, Melchinger AE, Frisch M, Maurer HP, Heckenberger M, Reif JC (2005) Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theor Appl Genet 111:723–730

Tardieu F, Tuberosa R (2010) Dissection and modelling of abiotic stress tolerance in plants. Curr Opin Plant Biol 13:206–212. doi:10.1016/j.pbi.2009.12.012

Trebbi D,Maccaferri M, de Heer P, Sorensen A, Giuliani S, Salvi S, Sanguineti MC,Massi A, van der Vossen EAG, Tuberosa R (2011) High-throughput SNP discovery and genotyping in durum wheat (Triticum durum Desf.). Theor Appl Genet 123:555–569. doi:10.1007/s00122-011-1607-7

Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3:347. doi:10.3389/fphys.2012.00347

Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M, Conti S (2002) Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. Ann Bot 89:941–963

Tuberosa R, Salvi S, Giuliani S, Sanguineti MC, Bellotti M, Conti S, Landi P (2007) Genome-wide approaches to investigate and improve maize response to drought. Crop Sci 47(Supplement 3):S120–S141. doi:10.2135/cropsci2007.04.0001IPBS

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1102. doi:10.1038/ng.2725

van Poecke RMP, Maccaferri M, Tang J, Truong HT, Janssen A, van Orsouw NJ, Salvi S, Sanguineti MC, Tuberosa R, van der Vossen EAG (2013) Sequence-based SNP genotyping in durum wheat. Plant Biotech J 11:809–817. doi:10.1111/pbi.12072

Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVS, Rebetzke GJ, Kirkegaard JA, Christopher J, WattM(2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63:3485–3498. doi:10.1093/jxb/ers111

Wasson AP, Rebetzke GJ, Kirkegaard JA, Christopher J, Richards RA, Watt M (2014) Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. J Exp Bot. doi:10.1093/jxb/eru250

Watt M, Moosavi S, Cunningham SC, Kirkegaard JA, Rebetzke GJ, Richards RA (2013) A rapid, controlled-environment seedling root screen for wheat correlates well with rooting depths at vegetative, but not reproductive, stages at two field sites. Ann Bot 112:447–455. doi:10.1093/aob/mct122

White RG, Kirkegaard JA (2010) The distribution and abundance of wheat roots in a dense, structured subsoil implications for water uptake. Plant Cell Environ 33:133–148. doi:10.1111/j.1365-3040.2009.02059.x

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208. doi:10.1038/ng1702

Yu G-R, Zhuang J, Nakayama K, Jin Y (2007) Root water uptake and profile soil water as affected

by vertical root distribution. Plant Ecol 189:15–30. doi:10.1007/s11258-006-9163-y

Zhu J, Ingram PA, Benfey PN, Elich T (2011) From lab to field, new approaches to phenotyping root system architecture. Curr Opin Plant Biol 14:310–317. doi:10.1016/j.pbi.2011.03.020

FIGURE

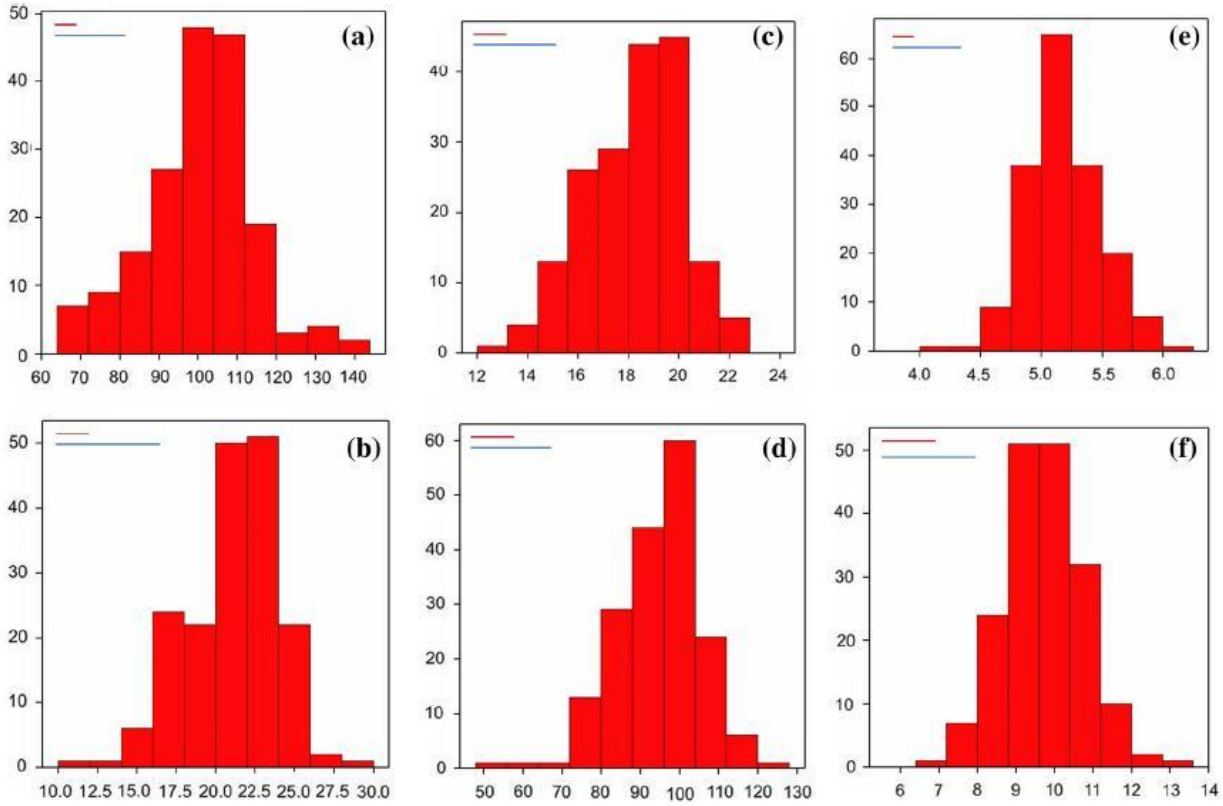

Fig. 1 Frequency distribution of the RSA traits measured in the collection of 183 elite lines of durum wheat at the four-leaf growth stage. The red line at the top of each graph represents the standard deviation calculated on the check cultivar Meridiano. The blue line represents the LSD ($P < 0.05$) between accessions

(a) Seminal root angle (SRA, °).

(b) Primary root length (PRL, cm).

(c) Average root length (ARL, cm).

(d) Total root length (TRL, cm).

(e) Total root number (TRN, no.).

(f) Shoot length (SL, cm). (Color figure online)

TABLES

Table 1 Mean, maximum and minimum values, ANOVA results and repeatability for the RSA traits and shoot length investigated at the four-leaf stage in seedlings of 183 durum wheat elite accessions

	SRA (°)	PRL (cm)	TRL (cm)	ARL (cm)	TRN (no.)	SL (cm)
Mean	100	21.1	94.6	18.2	5.18	9.70
Max	147	32.9	144.7	26.0	6.46	16.31
Min	48	13.8	52.8	12.0	4.01	7.20
Check (mean value)[(a)]	105	20.0	88.6	17.2	5.11	9.82
P accessions [(b)]	**	**	**	**	**	**
P replicates [(c)]	ns	ns	ns	ns	**	**
CV (%)	12.0	17.0	13.0	11.5	6.1	13.0
h^2 (%)	72.8	48.6	59.5	61.8	67.0	55.3
LSD ($P < 0.05$)	18.2	5.8	20.0	3.4	0.51	2.04

SRA seminal root angle, *PRL* primary root length, *TRL* total root length, *ARL* average root length, *TRN* total root number, *SL* shoot length, *CV* coefficient of variation, h^2 repeatability (mean basis), *LSD* least significant difference ($P < 0.05$)

[a] Meridiano, reference check line

[b] Significance of the difference between accessions

[c] Significance of the difference between replicates. ns = non significant

[*] $P < 0.05$; ** $P < 0.01$

Table 2 Correlation coefficient values and level of significance between root seminal traits (RSA) measured at the four leaf stage with the agronomic traits measured in 15 field trials (see ‘‘Materials and Methods’’), classified according to their average productivity levels, i.e., low, medium and high yielding environments (LYE_s^M, MYE_s^M and HYE_s^M, respectively), and with the general mean of environments (GME)

Agronomic traits	GY				TKW			
	LYE_s^M	MYE_s^M	HYE_s^M	GME_s	LYE_s^M	MYE_s^M	HYE_s^M	GME_s
Correlation values among RSA traits with GY and TKW								
RSA traits								
SRA		[1][a]				−0.23** [2]	−0.21** [3]	−0.21*
PRL			[1]			[1]		
TRL	[1]		[1]					
ARL			[1]					
TRN	0.24**[1]			0.18*				
SL			[1]					

Agronomic traits	KPSM				TW			
	LYE_s^M	MYE_s^M	HYE_s^M	GME_s	LYE_s^M	MYE_s^M	HYE_s^M	GME_s
Correlation values among RSA traits with KPSM and TW								
RSA traits								
SRA		0.23** [3]	0.24** [3]	0.23**	-0.20*[1]	−0.26** [3]	[1]	−0.22**
PRL		[1]				[1]	[1]	
TRL	[1]							
ARL	[1]		[1]				[1]	
TRN	0.18 [1]					[1]		
SL		[1]						

Agronomic traits	PH				PdL			
	LYE_s^M	MYE_s^M	HYE_s^M	GME_s	LYE_s^M	MYE_s^M	HYE_s^M	GME_s
Correlation values among RSA traits with PH and PdL								
RSA traits								
SRA		[1]	[1]		−0.21*[1]	[1]	−0.19* [1]	−0.20*
PRL	[1]							
TRL								
ARL								
TRN								
SL		0.19* [1]	0.21** [3]	0.20*				

Traits are abbreviated as follows: *GY* grain yield, *TKW* thousand kernel weight, *KPSM* kernels per square mt, *TW* test weight, *PH* plant height, *PdL* peduncle length, *SRA* seminal root angle, *PRL* primary root length, *TRL* total root length, *ARL* average root length, *TRN* total root number, *SL* shoot length

[a] The numbers reported in square brackets indicate in how many environments of each category a significant correlation was detected

Table 3 QTLs with significant concurrent effects on RSA and agronomic traits and number of environments where the association was significant is reported. Peak positions related to a durum consensus map (see ‘‘Materials and Methods’’ for details)

QTL	Trait	Marker	Chrom.	Peak	-log10	R^2	% effect	GY No. envs	TKW No. envs	KPSM No. envs	TW No. envs	PH No. envs	PdL No. envs
QSRA$_1$-1B	SRA	wPt-0655-1B	1B	4.4	2.38	5.31	10.17	1 LYE	-	-	4 (1 LYE, 1MYE, 2HYE)	-	-
QPRL$_1$-1B	PRL	gwm124-1B-a2	1B	109	2.32	6.18	7.91	-	-	-	LYEs, MYEs, GMEs	-	-
QARL$_1$-2A	PRL	cfa2263-2A-a2	2A	73.7	2.13	5.56	-13.29	-	-	7 (3 LYEs, 2 MYEs, 2 HYEs), GMEs	2 (1 LYE, 1 MYE), MYEs, GMEs	-	MYEs
	ARL	cfa2263-2A-a2		73.7	3.25	9.41	-13.46						
	TRL	cfa2263-2A-a2		73.7	2.78	7.33	-14.12						
QPRL$_2$-2B	PRL	barc183-2B-a2	2B	67.1	2.88	5.83	-12.80	2 (1 LYE, 1 HYE)	-	-	2 HYE, MYEs, GMEs	-	-
	ARL	barc183-2B-a2		67.1	2.56	5.25	-9.29						
	TRL	barc183-2B-a2		67.1	2.12	4.21	-9.31						
QSL$_1$-3A	SL	wmc388-3A-a2	3A	20.2	2.08	4.14	-5.25	-	1 HYE	1 HYE	1 HYE, GMEs	-	-
QTRN$_1$-3A	TRN	wmc428-3A-a6	3A	48.4	2.23	5.10	-5.41	-	5 (1 MYE, 4 HYE), HYEs	8 (1 LYE, 3 MYE, 4 HYE), MYEs, HYEs, GMEs	2 (1 MYE, 1 HYE), HYEs	6 (2 MYE, 4 HYE)	2 (1 MYE, 1 HYE)
QSRA$_2$-3A	SRA	barc1177-3A-a1	3A	154	2.03	4.86	7.39	-	-	-	3 (2 MYE, 1 HYE)	-	-
QPRL$_3$-4A	PRL	wPt-2946-4A	4A	88.3	2.19	4.47	10.29	-	1 HYE	2 (1 MYE, 1 HYE)	-	-	-
QSRA$_3$-4B	SRA	gwm888-4B-a2	4B	29.6	2.18	4.73	-6.28	3 (2 LYE, 1 MYE), LYEs, MYEs, GMEs	1 MYE	1 MYE, LYEs	-	3 (1 MYE, 2 HYE), HYEs	1 LYE

Table 3 continued …

QTL	Trait	Marker	Chrom.	Peak	-log10	R^2	% effect	GY No. envs	TKW No. envs	KPSM No. envs	TW No. envs	PH No. envs	PdL No. envs
$QTRN_2$-4B	TRN	gwm6-4B-a6	4B	85.4	2.04	5.59	3.54	1 HYE	3 (1 MYE, 2 HYE)	3 (2 MYE, 1 HYE)	-	-	-
$QSRA_4$-6A	SRA	gwm427-6A-a4	6A	128	3.13	7.74	-10.00	-	5 (1 LYE, 2 MYE, 2 HYE), LYEs, HYEs	-	1 LYE, LYEs	-	-
$QTRL_1$-6B	TRL	wPt-7343-6B	6B	14.3	2.13	5.32	6.21	GMEs	LYEs	1 MYE, MYEs	-	3 (1 MYE, 2 HYE), MYEs, HYEs, GMEs	4 (1 LYE, 1 MYE, 2 HYE), HYEs, GMEs
$QSRA_5$-6B	SRA	wPt-6594-6B	6B	21.5	2.31	4.59	6.16	2 HYE	LYEs	-	2 MYE	-	-
$QSRA_6$-6B	SRA	gwm1486-6B-a5	6B	153	3.26	7.43	-8.81	2 (1 LYE, 1 HYE), HYEs	-	1 LYE	-	-	3 (1 LYE, 1 MYE,1 HYE), GEMs
$QARL_2$-7B	ARL	gwm333-7B-a5	7B	79.1	2.18	4.67	-10.79		4 (1 LYE, 2 MYE, 1 HYE)	1 HYE, MYEs, GMEs	2 (1 MYE, 1 LYE)	-	LYEs

Traits are abbreviated as follows: *GY* grain yield, *TKW* thousand kernel weight, *KPSM* kernels per square mt, *TW* test weight, *PH* plant height, *PdL* peduncle length, *SRA* seminal root angle, PRL primary root length, *TRL* total root length, *ARL* average root length, *TRN* total root number, *SL* shoot length

For each agronomic trait, the number (no. envs) and category of environments (i.e., low, medium or high-yielding environment: LYE, MYE and HYE, respectively) in which the QTL was detected is reported. Additionally, the acronyms **LYEs**, **MYEs**, **HYEs** and **GMEs** (in bold) indicate when an RSA QTL co-located with a QTL for agronomic traits based on the analysis of the mean values in low, medium and high-yielding environments and across all 15 environments, respectively. The allele effect has been computed as detailed in the last sentence of ‘‘Materials and Methods’’

CHAPTER 4. LINKAGE MAPPING FOR ROOT SYSTEM ARCHITECTURE IN DURUM WHEAT MERIDIANO × CLAUDIO MAPPING POPULATION

4.1 ABSTRACT

Root system architecture (RSA) is of great agronomic importance and it has emerged in recent years as an important focus for plant genetics and breeding study. The genetic basis of variation for RSA traits were investigated using a population of 176 recombinant inbred lines (RILs) derived from the cross between two Italian elite durum wheat cvs. Meridiana and Claudio, in order to identify QTLs for RSA and compare their overlaps with other QTLs identified in other experiments and environments. The following seedling-stage RSA and seed traits were: seminal root angle, primary root length, total root length, root number, thousand kernel weight, shoot length, root and shoot dry weight. The results indicated a wide range of variation for RSA traits. The largest heritability was observed for thousand kernel weight (78.6%) and seminal root angle (65.4%). In total, 48 novel QTLs for RSA traits were identified on all chromosomes, with the exception of chromosome 4A. Both parents contributed favorable alleles at QTLs. Among the considered RSA traits, seminal root angle appears the most promising for undertaking further studies on the role of RSA traits. The most important QTLs for seminal root angle identified in this study mapped on chromosomes 4B and 6B.

Key words: Durum wheat, Root system architecture, QTL, Linkage mapping

4.2 INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. *durum*, 2n = 4x = 28; AABB genomes) is an important crop for the agriculture and economy of Mediterranean countries including Italy, Spain, France, Greece and the West Asian and North African (WANA) countries, where this cereal has received special attention as an important commodity throughout history (Elias and Manthey, 2005; Habash et al. 2009; Azizi et al. 2014).

Throughout its life cycle, root system architecture (RSA) is finely tuned to the requirements of the whole plant. Roots play several essential roles in the plant life cycle, including anchoring to the soil, mechanical support to stems, uptake of nutrients and water, sensing of variation in water and nutrient levels and others (Osmont et al. 2007; Smith and De Smet, 2012; Orman-Ligeza et al. 2013). Because of the tight connection between roots and soil, RSA plays a key role in environmental stress tolerance and are by definition extremely developmentally and physiologically plastic (Grossman and Rice, 2012). However, RSA traits are also characterized by constitutive genetic inheritance components which may enable to predict the root phenotypes based on genetic information. Additionally, in some cases, expression of RSA at adult plant stage are correlated with RSA at seedling stage, making highly valuable the genetic information gathered at the seedling stage.

Root system architecture (RSA) is of great agronomic importance and it has emerged in recent years as an important focus for plant genetics and breeding study. Although the key impediment to genetic analysis of cereal RSA has been the ability to study roots in situ, phenotypic observation protocols have been developed for adult plants under field conditions (Manschadi et al. 2006; Wojciechowski *et al*., 2009) and, more frequently, for young and/or adult plants grown in rizothrons under controlled environmental conditions (Bengough et al. 2004; Sanguineti et al. 2007; Liu et al., 2013).

Analyses of genetic factors contributing to RSA are however still very limited, partly because of the difficulty of observing the distribution of roots in field conditions, and partly because of the complexity of the effects of environmental conditions on root system architecture. Moreover, field screening is usually destructive and leads to a substantial loss of the geometry of the root (Nagel et al., 2009). In this respect, it has been reported that adult geometry of the root is strongly related to seminal root angle

(SRA), with deep-rooted wheat genotypes showing a narrower SRA, while genotypes with a shallower root system tend to grow their SRA wider (Manschadi et al., 2008). So, given that the trait measured in the early phases of plantlets' life might determine the root system later in the season, different systems have been adopted to allow early screening of high numbers of wheat plantlets for the investigation of RSA traits under controlled conditions (Zhu et al., 2005; Kubo et al, 2007; Sanguineti et al., 2007; Nagel et al., 2009; Yang et al., 2010; Ren et al., 2012; Bai et al., 2013; Christopher et al., 2013; Liu et al., 2013; Wasson et al. 2014). Among others, laboratory-based root culture systems have been developed to investigate root morphology, mainly at the seedling stage, e.g. in hydroponic systems, in glass or plastic rizothrons filled with soil, sand or artificial substrates, and paper systems. The paper culture system provides a high-throughput screening method to investigate RSA on large scale, albeit limited to young seedlings (Bai et al., 2013).

Quantitative trait loci (QTL) detection based on high-density molecular markers genetic maps and association mapping has improved the understanding of the complex genetic control of cereal root traits. Many of these studies have observed an overlap between QTL for root traits and those for nutrient uptake and productivity in maize (Tuberosa et al. 2002), wheat (An et al. 2006) and rice (Steele et al. 2007). However, for reported wheat QTLs, genome or gene-content sequence data is not currently available to allow confirmation of any relationship with root QTL in other crop species.

In common wheat, molecular mapping of root trait QTLs have been reported by a number of studies (Landjeva et al. 2008; Sharma et al. 2011; Hamada et al. 2012; Ren et al. 2012; Bai et al. 2013; Christopher et al. 2013; Liu et al. 2013). Collectively, these studies have demonstrated the presence of multiple QTLs for several major root traits, such as seminal root angle (SRA), primary root length (PRL), seminal root number (SRN), maximum root length (MRL), lateral root length (LRL), total root length (TRL), and root surface area (RSA). Furthermore, it has been generally realized that the development and morphological characteristics of seminal roots at seedling stage have important influences on the function of the root system in mature wheat plants. For example, the number of seminal roots has been found to correlate strongly with seed size and grain yield (MacKey 1979; Liu et al. 2013) and to

contribute significantly to water uptake (Manschadi et al. 2008) in common wheat. Thus, identifying and analyzing QTLs controlling seminal root traits will likely provide important information and resources for improving root function and its contribution to increase wheat yield.

However, the genetic mechanisms controlling root system architecture and function are still not well understood, especially in durum wheat, which possesses a complex and unsequenced tetraploid genome. Sanguineti et al. (2007) have reported the first results about genetic dissection of seminal root architecture in 57 elite durum wheat germplasm.

In accordance with this realization, we have previously carried out an association mapping study in 183 durum wheat elite accessions characterized at the seedling stage, in order to identify QTLs for root system architecture. The QTLs identified were compared with QTLs detected for grain yield and its component traits, plant height and peduncle length measured in a previous study where the same accessions were evaluated in 15 field trials. Out of the 48 QTLs detected for RSA, fifteen overlapped with QTLs for agronomic traits and/or grain yield in two or more environments (Canè et al. 2014).

In this study RSA traits were evaluated on the recombinant inbred line (RILs) population from the cross between the cvs. Meridiano and Claudio as a focus for studies on root trait architecture in durum wheat in an elite biparental durum wheat population. This study therefore fully complements the RSA analysis and QTL mapping efforts based on the germplasm collection and as reported in Chapter 3 (and Canè et al. 2014).

4.3 MATERIALS AND METHODS

4.3.1 Plant material

The plant material utilized for this study was a population of 176 recombinant inbred lines (RILs) originally developed in collaboration between University of Bologna and Società Produttori Sementi (Bologna, Italy), from the cross between the two Italian elite durum wheat cvs. Meridiano (Simeto/WB881//Duilio/F21) and Claudio (CIMMYT' selection/Durango//IS139b/Grazia)(Maccaferri et al. 2011). Both cvs. are currently extensively cultivated across Southern Europe. Meridiano is a high-yielding

early to medium-early heading genotype with broad adaptation, whereas Claudio is medium-early to medium-late depending on the environments and show good adaptation to drought areas.

4.3.2 Root system architecture evaluation

In order to study the genetic bases of RSA, durum wheat seedlings were grown in a randomized complete block design with two replications. The paper culture system at RSA traits was evaluated according to a protocol first described by Bengough et al. (2004) then modified by Sanguineti et al. (2007) and improved in the present work. In particular, for each genotype (RILs), 15 seeds were prepared and weighted exactly. Then, selected seeds were moved in the pre-germination step, in Petri dishes with a wet filter paper at 28 °C for 24 h. Then, seven germinated seeds for each genotype were placed on flat plastic rhizotrons covered with imbibed filter paper where seedlings were let to grow per one week, in the dark, at constant temperature of 25°C. Five well developed and homogeneous seedlings were utilized for phenotypic scoring. Examples of images of the root apparatus are shown in Fig. 1.

Due to the high number of the genotypes under evaluation, the accessions were operatively divided into sets of 40 accessions each (blocks), in order to be included in the ANOVA analysis to account for differences among the different sets with two parents cvs. Meridiano and Claudio parents were also included in every set as checks. Seventeen root system architecture traits were collected and traits, abbreviations and explanations are shown in Table 1. All traits were manually measured and/or recorded by plantlets' images using the software SmartRoot® (Lobet et al. 2011), after recording digital images of each seedling.

One of the most important traits that was measured in this study was seminal root angle (SRA), that is the angle of the root apparatus according to the following:
SRA (rad) = 2* ASIN [(x/2)/a]
where **x** and **a** measured by SmartRoot software (Fig. 1).

4.3.3 Molecular profiling

Phenotypic data collected from the RILs was subjected to ANOVA, adjusted for block effects and used as phenotypic data for QTL mapping with the goal of detecting

QTL associated between root system architecture traits. In total, 899 markers including 487 single nucleotide polymorphisms (SNP), 261 diversity array technology (DArT), 142 simple sequence repeat loci (SSR), and nine additional sequence tagged site (STS) markers were used for the molecular profiling of the 176 recombinant inbred lines (RIL) of durum wheat.

The markers were ordered according to a consensus map developed at the University of Bologna in the framework of an international cooperation for that purpose (Maccaferri et al. 2014). The mapping population, Meridiano × Claudio (MC RIL, Maccaferri et al. 2011) was developed by the University of Bologna in collaboration with Produttori Sementi Bologna SpA (Argelato, BO, Italy). Additional maps provided by international partners were used to assemble a common consensus map, used to order the markers available for genotyping the experimental materials herein presented. A linkage map including 27 linkage groups, for a total length of 2238.16 cM was assembled and marker grouping was performed using the independence LOD method with LOD threshold range from 2.0 to 10.0 (Maccaferri et al. in preparation). Mapping analysis were carried out in Joinmap v4.0 and Carthagene softwares.

4.3.4 Statistical analysis and QTL analysis

Frequency distributions of the phenotypic data were inspected to assess the consistency of data and to investigate the complexity of the genetic control of the traits. The analysis of variance (ANOVA) for each trait was performed using the software Genstat *V.15.* and RIL means were used to calculate Pearson's correlation coefficients of RSA traits. Heritability (h^2) was calculated on a mean basis across two replications according to the following:

$$h^2 = \frac{\sigma^2{}_G}{\sigma^2{}_G + \frac{\sigma^2{}_E}{r}}$$

where $\sigma^2{}_G$ and $\sigma^2{}_E$ represent the genotypic and the environmental components of the phenotypic variance, respectively, and r the number of replications. Heritability should be considered as being 'narrow sense' because the genetic variance included only the additive component and, possibly, the additive × additive epistatic interaction (Sanguineti, et al. 2007).

In total, 899 markers (487 SNPs, 261 DARTs, 142 SSRs and 9 STSs) was utilized for QTL analysis. Single-marker analysis using linear regression, composite interval mapping (CIM) and multiple interval mapping (MIM) were carried out in Windows QTL Cartographer version 2.5 (WINQTL) (Wang et al. 2007). Initially, single marker analysis was used to identify genetic markers significantly associated with phenotypic traits. Composite interval mapping (CIM) and multiple interval mapping (MIM) were then used to determine the most likely QTL positions and the LOD threshold for each trait. Final results from the more precise MIM analysis are reported.

The reported QTLs were identified as having a threshold LOD $\geq$ 2.0 through composite, and multiple interval mapping. Confidence intervals of QTL (CL) were calculated by the following formula (95% CI of each QTL):

$CI = 163/(N \times R^2)$,

where *N* is the number of lines in the mapping population and R^2 is the percentage of phenotypic variation explained by the identifyed QTLs (Liu et al. 2009).

4.4 RESULTS

4.4.1 Phenotypic analysis of root system architecture

A large range of variation was observed for all traits among RILs. In detail, TKW and SRA of the samples, ranged from 39.8 to 63.4 g, with mean value of 48.3 g and 68.3° to 128.5°, with mean value of 97.9°, respectively. TRL was the trait that showed the largest range of variation, from 95.8 to 142.39 cm, with a mean value of 122.5 cm and diamSR indicated the lowest range of variation, from 0.07 to 0.09 cm, with a mean value of 0.08 cm. Additionally, PRL ranged from 24.7 to 34.5 cm, with a mean value of 30.7 cm. The treatment effect and genetic variation within the 176 RIL population for RSA traits are reported in Table 2. Results of ANOVA showed significant differences among durum wheat RILs for all RSA traits except for SurPR, SurSR, VolPR and VolSR.

Coefficient of variation (CV) ranged from 5.0% for diamPR to 13.8% for RDW and heritability values ranged from 11.0% to 78.6% among traits and VolPR and TKW with the smallest and largest values, respectively (Table 2).

4.4.2 Correlations and distributions of the root system architecture traits

The correlation coefficients among RSA traits are reported in Table 3. Many traits correlated with one another. For conciseness, highly significant and negatively correlations were detected between TKW and most of the traits, including PRL (r = -0.45), TRL (r = -0.36), RDW (r = -0.35), SRL (r = -0.33), RSR (r = -0.26) and SL (r = -0.24). Also SRA showed negative correlation with TKW (r = -0.12).

RSR was high significantly and positively correlated with RDW (r = 0.47) and with SDW, albeit negatively (r = -0.52). Additionally, highly significant and positively correlations were identified between SDW and most of the traits, including SL (r = 0.70), RDW (r = 0.51), TRL (r = 0.36), SRL (r = 0.34), PRL (r = 0.30), SurSR (r = 0.26) and SurPR (r = 0.19).

Frequency distributions for RSA traits are shown in Fig. 2. All the RSA traits exhibited continuous variation in the RIL population with approximately normal distributions, indicating a polygenic control underlying these traits.

4.4.3 Identification of QTL for root system architecture traits

A total of 899 markers, were mapped to 27 linkage groups with a total map length of 2,238.16 cM. The linkage groups were assigned to durum wheat chromosomes 1A through to 7B.

For the seventeen RSA traits examined, 48 QTLs were identified using QTL Cartographer with a LOD score significant at $P \geq 2$. The results of QTL detection are presented in Table 4. In total, we identified 3 QTLs for TKW, 3 for SRA, 3 for TNR, 2 for R6, 3 for PRL, 3 for SRL, 3 for TRL, 2 for diamPR, 3 for diamSR, 3 for SurPR, 3 for SurSR, 3 for VolPR, 3 for VolSR, 3 for SL, 2 for RDW, 3 for SDW and 3 for RSR. The QTLs were identified on all chromosomes, except chromosome 4A and more than 38% of these QTLs were located on chromosomes 4B and 7A (21% and 17%, respectively). QTLs for several root traits co-mapped within a 15 cM interval. According to this, the 48 QTLs were organized into 12 QTL clusters and 36 QTLs for individual RSA traits. The QTLs belonging to QTL clusters most probably share the same genetic basis. Both parents contributed favorable alleles to the population, with

33 from Meridiano and 15 from Claudio. The QTL distribution in the genetic linkage map is reported in Fig. 3.

4.4.3.1 Identification of QTLs for thousand kernel weight

The QTLs associated with TKW were identified and detected on chromosomes 3B, 4B and 6A, respectively. The largest effect QTL for TKW was *QTKW3-6A*, flanked by a SNP marker IWB66509 on chromosome 6B with R^2 values 10.54% and a negative additive effect of -1.43. The other TKW associated QTL was located on chromosome 4B (*QTKW2-4B*). This QTL was related to a SSR marker wmc89a-4B with a negative additive effect of -1.27 and R^2 values 8.4%. The last TKW associated QTL, with the smallest effect was detected on chromosome 3B (*QTKW1-3B*), and flanked by a DART marker wPt-8686-3B. It had a negative additive effect of -1.08 with R^2 values 6.09%. The favorable allele at these QTLs was contributed by Claudio (Table 4 and Fig. 3).

Based on the presence of genetic variation and QTLs for TKW in the mapping population, before QTL analysis the RSA traits were subjected to covariance analysis using the TKW phenotypes and, in case of significance, the co-variated RSA phenotypes were used in the analysis.

4.4.3.2 Identification of QTLs for seminal root angle

The putative QTL associated with SRA were respectively identified on chromosomes 4B, 6B and 7A. The greatest effect QTL for SRA was *QSRA1-4B*, related to a SNP marker IWB71667b on chromosome 4B with R^2 values 10.39% and a negative additive effect (-3.23). Considering a negative additive effect at this QTL, Claudio contributed the allele that widened the root angle at this locus.

The second and third SRA associated QTLs were located on chromosome 6B (*QSRA2-6B*) and 7A (*QSRA3-7A*). These QTLs were flanked by SNP markers IWB27199 and IWB35428, with R^2 values 10.01% and 6.17%, respectively. Meridiano allele at these QTLs associated with positive additive effects (2.50 and 3.17) has contributed the plus SRA allele at these locus (Table 4 and Fig. 3).

4.4.3.3 Identification of QTLs for total number root and percent of 6th root

Among the five RSA-QTLs that were identified, three (*QTNR1-2A*, *QTNR2-2B* and *QTNR3-4B*) co-located for TNR, and two (*QR6-1-4B* and *QR6-2-6B*) were detected for R6.

The major QTLs for TNR and R6 were located on chromosome 4B (*QTNR3-4B* and *QR6-1-4B*), flanked by SNP markers IWB72368 and IWB9672 with R^2 values of 12.65% and 10.08%, respectively. Considering the positive additive effects of all five QTLs for TNR and R6, Meridiano contributed the favorable allele among them (Table 4 and Fig. 3). The percentage of plants with the sixth root (R6) varied from 0% (in 49 RILs) to 60% (i.e. presence of the sixth root in 60% of seedlings, on average).

4.4.3.4 Identification of QTLs for primary, seminal and total root length

Out of the nine RSA-QTLs that were detected, three (*QPRL1-6A*, *QPRL2-6B* and *QPRL3-7B*) co-located for PRL, three (*QSRL1-1B*, *QSRL1-2B* and *QSRL1-6B*) for SRL and three (*QTRL1-1B*, *QTRL2-3B* and *QTRL3-4B*) for TRL.

The largest effect of QTL for PRL was *QPRL2-6B*, related to a SSR marker wmc182-6B on chromosome 6B with R^2 value 7.05%. The SRL and TRL associated QTLs with the greatest effects were located on chromosome 1B (*QSRL1-1B* and *QTRL1-1B*) and flanked by a DART marker wPt-733882-1B with R^2 values of 10.25% and 11.85%, respectively. According to the results of additive effects, the favorable alleles in *QPRL2-6B*, *QSRL1-1B* and *QTRL1-1B* have been contributed by Claudio and the other QTLs contributed by Meridiano (Table 4 and Fig. 3).

4.4.3.5 QTLs for diameter, surface and volume of primary and seminal roots

Among the RSA-QTLs that were identified, two (*QdiamPR1-1A* and *QdiamPR2-7A*) co-located for diamPR, three (*QdiamSR1-1A*, *QdiamSR2-2A* and *QdiamSR3-7A*) for diamSR, three (*QSurPR1-4B*, *QSurPR2-6A* and *QSurPR3-7B*) for SurPR, three (*QSurSR1-1B*, *QSurSR2-4B* and *QSurSR3-7A*) for SurSR, three (*QVolPR1-1A*, *QVolPR2-6A* and *QVolPR3-7A*) for VolPR and three (*QVolSR1-1B*, *QVolSR2-4B* and *QVolSR3-7A*) for VolSR.

The diamPR and diamSR associated QTLs with the highest effects were located on chromosome 1A (*QdiamPR1-1A*) and 7A (*QdiamSR3-7A*) with R^2 values of 9.02%

and 8.79%, respectively. The favorable alleles of all QTLs associated with diamPR and diamSR were contributed by Meridiano.

The SurPR and SurSR associated QTLs with the largest effects were located on chromosome 6A (*QSurPR2-6A*) and 1B (*QSurSR1-1B*) with R^2 values of 7.49% and 11.65%, respectively. Except in, *QSurSR1-1B* that had a negative additive effect by Claudio, the favorable alleles in the other QTLs associated with SurPR and SurSR were contributed by Meridiano.

The VolPR and VolSR associated QTLs with the greatest effects were located on chromosome 7A (*QVolPR3-7A* and *QVolSR3-7A*) and flanked by a SNP marker IWB73246 with R^2 values of 6.51% and 10.99%, respectively. Except in *QVolSR1-1B* that had a negative additive effect by Claudio, the favorable alleles in the other QTLs associated with VolPR and VolSR were contributed by Meridiano (Table 4 and Fig. 3).

4.4.3.6 QTLs for shoot length

The QTLs associated with SL were identified and detected respectively on chromosomes 3A, 4B and 7A. The largest effect QTL for SL was *QSL2-4B*, related to a SNP marker IWB70674 on chromosome 4B with a positive additive effect of 0.32 and R^2 values 13.52%. The favorable allele at this QTL was contributed by Meridiano.

The second and third SL associated QTLs were located on chromosome 3A (*QSL1-3A*) and 7A (*QSL3-7A*). These QTLs were related by a SSR marker wmc505-3A and a SNP marker IWB73857 with R^2 values 9.16% and 6.56%, respectively. The Claudio allele at these QTLs associated with negative additive effects (-0.27 and -0.22) and contributed the favorable allele (Table 4 and Fig. 3).

4.4.3.7 QTLs for root and shoot dry weight and root shoot ratio

Among of the considering RSA-QTLs that were detected, two (*QRDW1-6A* and *QRDW2-7B*) co-located for RDW, three (*QSDW1-4B*, *QSDW2-5A* and *QSDW3-5B*) for SDW and three (*QRSR1-3B*, *QRSR2-3B* and *QRSR3-7A*) for RSR.

The QTL with the largest effect for RDW was located on chromosome 7B (*QRDW2-7B*), and related to a DART marker wPt-8040-7B with R^2 value 4.12%. The favorable allele at this QTL has been contributed by Meridiano.

The SDW and RSR associated QTLs with the greatest effects were located on chromosome 4B (*QSDW1-4B*) and 7A (*QRSR3-7A*) respectively, and flanked by SNP markers IWB13072 and IWB58109 with R^2 values of 10.51% and 7.27%, respectively. Considering a positive additive effects at these QTLs, Meridiano has contributed the favorable allele (Table 4 and Fig. 3).

4.4.3.8 RSA Features of QTL clusters

Among the QTLs detected for RSA traits, 31 QTLs for individual traits were organized in 12 main QTL clusters. QTL clusters are reported according to their map position in Fig. 3. In total, 12 main QTL clusters were identified on some of chromosomes, including chromosome 1A one QTL cluster, 1B two QTL clusters, 3B one QTL cluster, 4B three QTL clusters, 6A one QTL cluster, 6B two QTL clusters, 7A one QTL cluster and 7B one QTL cluster.

The largest coincidence between individual traits were observed in QTL cluster located on chromosome 7A for five traits including diamPR, diamSR, SurSR,VolPR and VolSR. This QTL cluster flanked by SNP marker IWB73246. Additionally, coincidence between QTLs for SurSR and VolSR was observed for a few QTLs, namely on chromosomes 1B (18 cM) and 4B (113 cM). These QTLs were related to DART markers tPt-5413-1B and wPt-663949-4B, respectively.

Coincidence between QTLs for diamPR and VolPR was observed for only one QTL region (chromosome 1A, 1 cM). Another QTL cluster for primary root features was located on chromosome 6A (VolPR, PRL, SurPR, in coincidence to wPt-732328-6A). Similarly, coincidence between QTLs for TRL and SRL was observed for only one QTL region (chromosome 1B, 55 cM).

In this study, QTLs forSRA were located on two QTL clusters on chromosomes 4B (96 cM) and 6B (191 cM) with R^2 values of 10.39% and 6.17%, respectively. *QSRA1-4B, QSurPR1-4B* and *QTRL3-4B* co-located on the QTL cluster on chromosome 4B. *QSRA2-6B* and *QR6-2-6B* co-located on QTL cluster of chromosome 6B.

4.5 DISCUSSION

The importance of roots in water and nutrient acquisition has been acknowledged by scientists for a long time however RSA traits have hardly entered breeding programs so far (Den Herder et al. 2010; Cao et al. 2014). Indeed, mapping QTL for root system architecture traits will help breeders to select root traits desirable for efficient acquisition of water and nutrients from soils. The aim of the current study was to identify the genetic regions controlling root system architecture in the recombinant inbred line population from the cross between the cvs. Meridiano and Claudio.

The RIL durum wheat tested in this research showed a range and coefficient of variation suitable for further investigation. Most traits showed normal distributions among RILs suggesting the presence of polygenic control. High heritability values were obtained for TKW (78.6%) and SRA (65.4%) prompting to further genetic investigations. Other RSA traits showed medium to good heritability values (from 11.0% to 64.2%), allowing us to find QTLs for RSA traits.

The results of correlation coefficients indicate that highly significant and negatively correlations were detected between TKW and the most of the traits, including PRL, TRL, RDW, SRL, RSR and SL. These observations are in line with the results of Sanguineti et al. (2007) who reported kernel weight (KW) as negatively correlated with PRL, TRL and SL.

RSR highly significantly correlated with RDW (positively), and with SDW (negatively). These findings are in agreement with Bai et al. (2013) on 199 lines of DH population in wheat seedlings. Among the investigated root traits, SRA showed negative correlation with TKW. We have previously reported negative correlations between SRA and TKW in three environmental classes (Chapter 3 and Canè et al. 2014).

In this study, 48 quantitative trait loci (QTL) were identified for 17 RSA traits across a RIL population from the cross between two durum wheat cvs. Meridiano and Claudio. The QTLs were identified on all chromosomes, except on chromosome 4A. Both parents contributed favorable alleles to QTLs. QTLs mapped in 12 main QTL clusters (including more QTLs for more than one trait) and 31 QTLs for individual traits.

SRA (collected as the widest angle of the root apparatus) appears a trait of particular interest in both durum and bread wheat. Accordingly with Christopher et al. (2013), the angle of roots at their emergence from the seeds could be a good proxy for deep rooting potential. Roots with narrower root angle would enable the root system to grow deeper in the soil since root growth would direct downwards. If this prediction will be confirmed, this would be a good example of a breeding-useful correlation between an early expressed traits and the finally expressed important agronomic trait. (Kato et al. 2006; Omori and Mano. 2007; Manschadi et al. 2008; Christopher et al. 2013).

In rice, Kato et al. (2006) indicated that a nodal root angle can serve as a useful tool for rough estimation of vertical root distribution. In maize, Omori and Mano. (2007) evaluated variation in nodal root angle in two sets of F2 populations and reported a QTL related to root angle to be located on maize chromosome 7. Manschadi et al. (2008) demonstrated that selection for root growth angle may help to identify genotypes with root system architecture adapted to drought tolerance of wheat varieties. Additionally, Christopher et al. (2013) identified QTLs for root angle in a population developed from bread wheat and reported the QTLs associated with seminal root angle detected on chromosomes 2A, 3D, 6A and 6B. Sanguineti et al. (2007) have reported the first results about spread of root angle in 57 elite durum wheat germplasm and identified QTLs related to root angle to be located on chromosomes 3A, 4A, 5B and 6A.

In the present study, we successfully identified three QTLs for SRA in Meridiano × Claudio population that are located on chromosomes 4B, 6B and 7A (*QSRA1-4B*, *QSRA2-6B* and *QSRA3-7A*; Table 4). Analysis of genetic effect showed that both parents, Meridiano and Claudio, contributed favorable alleles. Detection of these QTLs further show that they are still segregating in cultivars adapted to the target production region and could potentially be responsive to the conventional or marker-assisted selection. In accordance with these results, we have previously investigated association mapping for root architectural traits in a set of 183 durum wheat seedlings and identified six QTLs for seminal root angle (SRA) on chromosomes 1B, 3A, 4B, 6A and 6B (Canè et al. 2014). According to these two results, the coincidence of chromosomes for SRA observed on chromosomes 4B and 6B. Based on the results

herein presented and our previously results, it is clear that two SRA controlled genes were located most likely on chromosomes 4B and 6B, whose effects were validated on a panel of genotypes independent from the Meridiano × Claudio population.

In our study, an important fraction (>38%) of identified RSA QTLs located on chromosomes 4B and 7A. Chromosome 4B had the largest number of QTLs (ten) including TKW, SRA, TNR, R6, TRL, SurPR, SurSR, VolSR, SL and SDW QTLs. At just two QTLs Claudio provided the favorable allele while for all others QTLs the positive allele originated from Meridiano. Eight QTLs were found on chromosome 7A for traits such as SRA, diamPR, diamSR, SurSR,VolPR, VolSR, SL and RSR. One SL QTL (*QSL3-7A*) was characterized by a favorable additive effect of Claudio's allele, while Meridiano contributed the favorable allele at all other QTLs. The alternation of favorable alleles originating from either Claudio or Meridiano at different QTLs for the same traits likely explains the commonly observed transgression of RIL values over parental phenotypic values.

Finally, we observed an intriguingly high concentration of QTLs on chromosome 7A near marker IWB73246. Traits with QTLs in this region were diamPR, diamSR, SurSR,VolPR and VolSR. We hypothesized that a single gene with pleiotropic effects underpins this QTL.

4.6 CONCLUSIONS

In the present study, a root phenotyping protocol based on paper culture system coupled with software-assisted image analysis enabled us to investigate variation for RSA traits at the seedling stage in a RIL population of durum wheat. Most RSA traits showed normal distribution suggesting that traits expression was under highly polygenic control. However, significant differences were identified among lines for most of traits indicating the presence of genetic variation potentially exploitable in breeding. These results are noteworthy especially considering that the tested materials were derived from the cross of highly productive elite Italian cvs, notoriously considered depleted in genetic variability. Among the considered RSA traits, seminal root angle (the angle of spread of the root apparatus) appeared the most promising for further investigations aimed at testing the implications of root angle variation on yield under stress conditions and/or gene cloning. In this direction, our study enabled us to

map two major QTLs controlling seminal root angle on chromosomes 4B and 6B. For these two QTLs, along with others, we provided molecular markers associated to the favorable allele.

4.7 REFERENCES

An DG, Su JY, Liu QY, Li B, Jing RL, Li JY, Li ZS (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (Triticum aestivum L.). Plant Soil 284:73–84

Azizi L, Mohammadi R, Etminan A, Shooshtari L (2014) Molecular discrimination of the advanced breeding lines of durum wheat (Triticum turgidum L.) based on inter simple sequence repeat markers. International Journal of Biosciences 5:12, 130-135

Bai C, Liang Y, Hawkesford MJ (2013) Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. J Exp Bot 64: 1745–1753

Belaid A (2000) Durum wheat in WANA: production, trade and gains from technological change. Proceedings of the seminar 'Durum wheat improvement in the Mediterranean region: new challenges. Zaragoza, Spain, 12–14 April 2000. Options Me´diterrane´ennes, Se´rie A, Se´minaires Me´diterrane´ens, 40, 35–49

Bengough AG, Gordon DC, Al-Menaie H, Ellis RP, Allan D, Keith R, Thomas WTB, Forster BP (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant and Soil, 262: 63–70

Canè MA, Maccaferri M, Nazemi G, Salvi S, Francia R, Colalongo C, Tuberosa R (2014) Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. Mol Breed 34:1629-1645

Cao P, Ren Y, Zhang K, Teng W, Zhao X, Dong Z, Liu X, Qin H, Li Z, Wang D, Tong Y (2014) Further genetic analysis of a major quantitative trait locus controlling root length and related traits in common wheat. Molecular Breeding 33: 975-985

Christopher J, Christopher M, Jennings R, Jones S, Flecher S, Borrell A, Manschadi AM, Jordan D, Mace E, Hammer G (2013) QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum*) with contrasting adaptation to water-limited environments. Theor Appl Genet 126: 1563–1574

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600–607

Elias EM, Manthey FA (2005) End products. In: Royo C, Nachit MM, Fonzo N, Araus JL, Pfeiffer WH, Slafer GA, editors. Durum wheat breeding: current approaches and future strategies. New York: Food Products Press; pp. 939–962

Grossman JD, Rice KJ (2012) Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. Evolutionary Applications 5: 850-857

Habash DZ, Kehel Z, Nachit M (2009) Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J Exp Bot 60: 2805–2815

Hamada A, Nitta M, Nasuda S, Kato K, Fujita M, Matsunaka H, Okumoto Y (2012) Novel QTLs for growth angle of seminal roots in wheat (Triticum aestivum L.). Plant Soil 354:395–405

Kato Y, Abe J, Kamoshita A, Yamagishi J (2006) Genotypic variation in root growth angle in rice (Oryza sativa L.) and its association with deep root development in upland fields with different water regimes. Plant Soil 287:117–129. doi:10.1007/s11104-006-9008-4

Kubo K, Elouafi I, Watanabe N, Nachit MM, Inagaki MN, Iwama K, Jitsuyama Y (2007) Quantitative trait loci for soil-penetrating ability of roots in durum wheat. Plant Breeding Volume 126, Issue 4, pages 375–378

Landjeva S, Neumann K, Lohwasser U, Bo¨rner A (2008) Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biol Plant 52:259–266

Liu S, Marla D.H, Carl A, Griffey LM, Anne LM (2009) Meta-analysis of QTL associated with fusarium head blight resistance in wheat. Crop Sci. 49:1955–1968

Liu X, Li R, Chang X, Jing R (2013) Mapping QTLs for seedling root traits in a doubled haploid wheat population under different water regimes. Euphytica: 189: 51–66.

Lobet G, Pages L, Draye X (2011) A novel image analysis toolbox enabling quantitative analysis of Root System Architecture. Plant Physiol 157: 29–39

Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357. doi:10.1093/aob/mcs293

Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. J Exp Bot. doi:10.1093/jxb/eru162

Maccaferri M, Ratti C, Rubies C, Vallega V, Demontis A, Stefanelli S, Tuberosa R, Sanguineti MC (2011) Resistance to Soil-borne cereal mosaic virus in durum

wheat is controlled by a major QTL on chromosome arm 2BS and minor loci. Theor Appl Genet 123:527–544

Maccaferri M, Cane MA, Salvi S, Noli E, Colalongo C,Massi A, Clarke F, Pozniak C, Korol A, Fahima T, Dubcovsky J, Xu S, Karsai I, Knox R, Clarke J, Tuberosa R (2014) A consensus framework map of durum wheat (Triticum durum Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. BMC Genomics 15:873

MacKey J (1979) Genetic potentials for improved yield. Acta Agron Hung 28:121–143

Manschadi AM, Christopher J, Devoil P, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. Funct Plant Biol 33:823–837

Manschadi AM, Hammer GL, Christopher JT, deVoil P (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (Triticum aestivum L.). Plant and Soil 303:115–129

Manschadi AM, Christopher JT, Hammer GL, Devoil P (2010) Experimental andmodelling studies of drought-adaptive root architectural traits in wheat (Triticum aestivum L.). Plant Biosyst 144:458–462. doi:10.1080/11263501003731805

Morancho J (2000) Production and marketing of durum wheat worldwide. Proceedings of the seminar ‘Durum wheat improvement in the Mediterranean region: new challenges. Zaragoza, Spain, 12–14 April 2000. Options Me´diterrane´ennes, Se´rie A, Se´minaires Me´diterrane´ens, 40, 35–49

Nagel KA, Kastenholz B, Jahnke S, van Dusschoten D, Aach T, Mühlich M, Truhn D, Scharr H, Terjung S, WalterA, Schurr U (2009) Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. Funct Plant Biol 36: 947–959

Omori F, Mano Y (2007) QTL mapping of root angle in F2 populations from maize ‘B73’ × teosinte ‘Zea luxurians’. Plant Root 1:57–65. doi:10.3117/plantroot.1.57

Orman-Ligeza B, Parizot B, Gantet PP, Beeckman T, Bennett MJ, Draye X (2013) Post-embryonic root organogenesis in cereals: branching out from model plants. Trends Plant Sci. 18: 459–467

Osmont KS, Sibout R, Hardtke CS (2007) Hidden branches: developments in root

system architecture. Annu Rev Plant Biol 58:93–113

Ren Y, He X, Liu D, Li J, Zhao X, Li B, Tong Y, Zhang A, Li Z (2012) Major quantitative trait loci for seminal root morphology of wheat seedlings. Mol Breed 30:139–148

Sanguineti MC, Li S, Maccaferri M, Corneti S, Rotondo F, Chiari T, Tuberosa R (2007) Genetic dissection of seminal root architecture in elite durum wheat germplasm. Ann Appl Biol 151: 291-305

Sharma S, Xu S, Ehdaie B, Hoops A, Clsoe TJ, Lukaszewski AJ, Waines JG (2011) Dissection of QTL effects for root traits using a chromosome arm-specific mapping population in bread wheat. Theor Appl Genet 122:759–769

Smith S, De Smet I (2012) Root system architecture: insights from Arabidopsis and cereal crops. Philos Trans R Soc Lond B Biol Sci. 367:1441-1452

Steele KA, Virk DS, Kumar R, Prasad SC, Witcombe JR (2007) Field evaluation of upland rice lines selected for QTLs controlling root traits. Field Crop Res 101:180–186

Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Molecular Biology 48: 697-712

Wang S, Basten CJ, Zeng ZB (2007) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Wasson AP, Richards RA, Chatrath R, Misra SC, Sai Prasad SV, Rebetzke GJ, Kirkegaard JA, Christopher J, Watt M (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. Journal of Experimental Botany 63- 9:3485–3498. doi:10.1093/jxb/ers111

Wasson AP, Rebetzke GJ, Kirkegaard JA, Christopher J, Richards RA, Watt M (2014) Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. J Exp Bot. doi:10.1093/jxb/eru250

Wojciechowski T, Gooding MJ, Ramsay L, Gregory PJ (2009) The effects of dwarfing genes on seedling root growth of wheat. Journal of Experimental Botany 60, 2565–2573

Yang M, Ding G, Shi L, Feng J, Xu F, Meng J (2010) Quantitative trait loci for root

morphology in response to low phosphorus stress in Brassica napus. Theoretical and Applied Genetics 121, 181–193

Zhu J, Kaeppler SM, Lynch JP (2005) Mapping of QTL controlling root hair length in maize (Zea mays L.) under phosphorus deficiency. Plant and Soil 270, 299–310

FIGURES

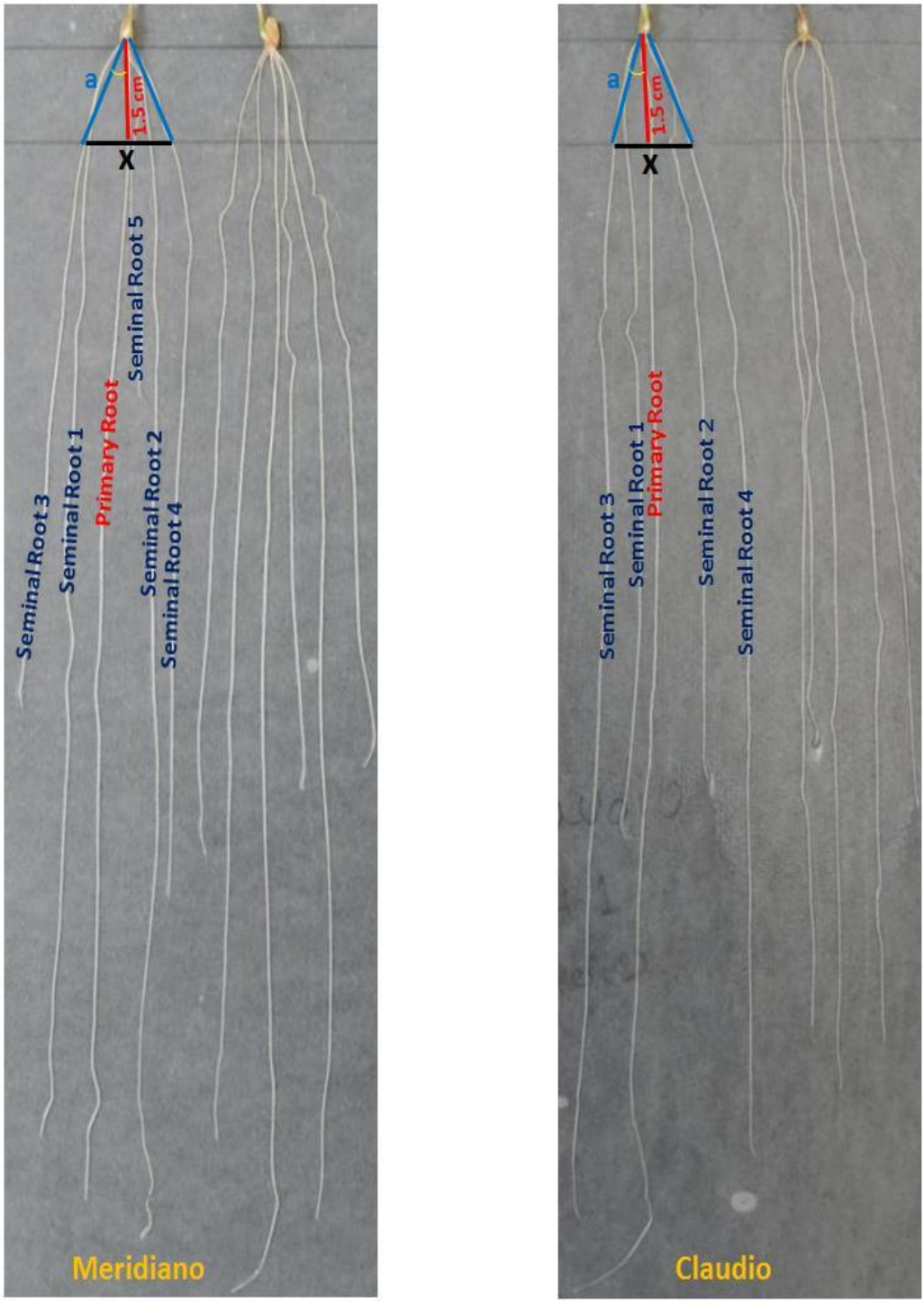

Fig. 1 Examples of images (digital photos) of the root system architecture in seedlings of the two parents (Meridiano and Claudio) of the cross population utilized in this study. Commonly, durum wheat has five or six roots, including a primary root and four or five seminal roots. The trait 'seminal root angle' (SRA), measured as the widest angle of the root apparatus is illustrated

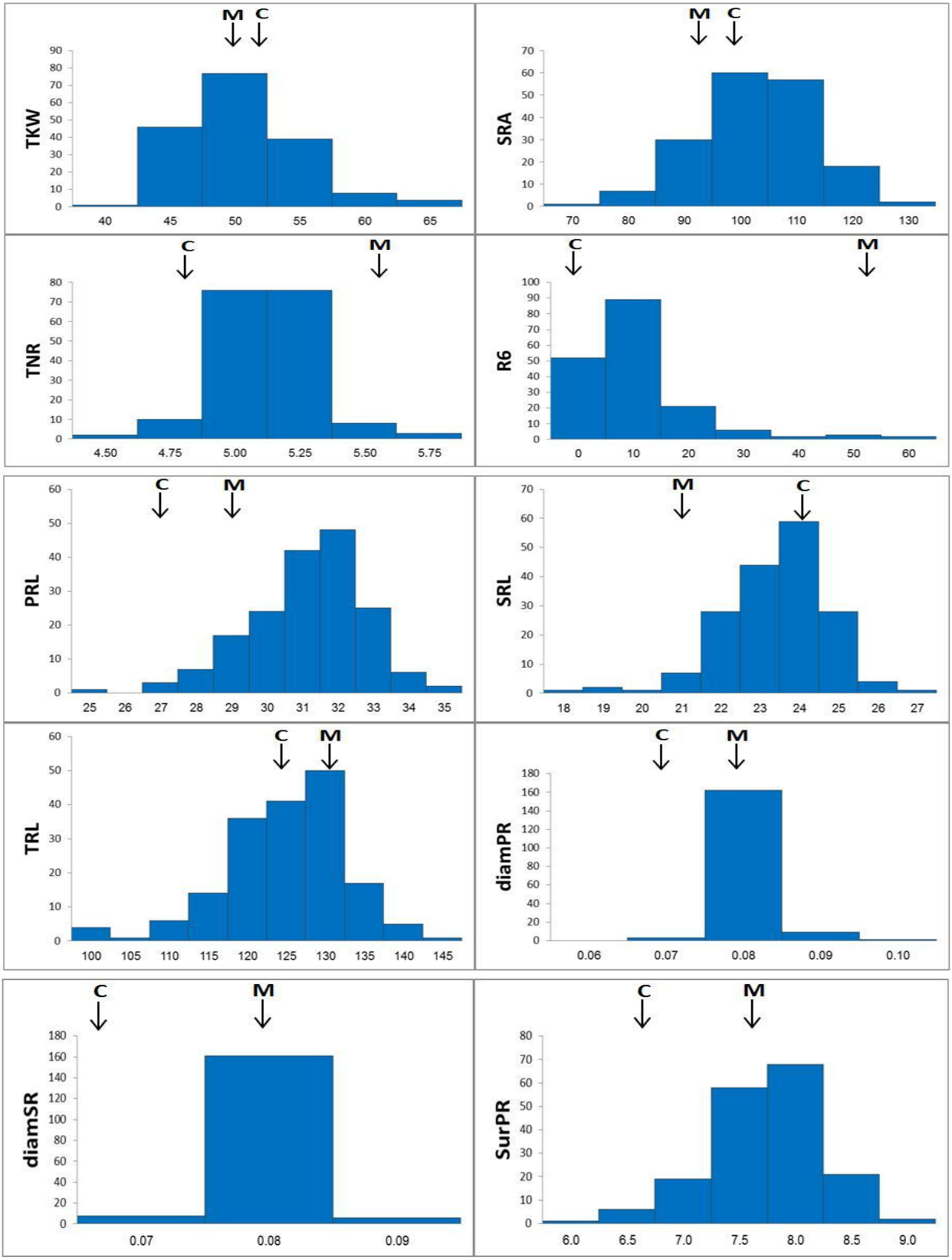

Fig. 2 Frequency distribution for the RSA traits in 176 Meridiano x Claudio RILs collected at seedling stage. Arrows indicate mean values for Meridiano (M), and Claudio (C)

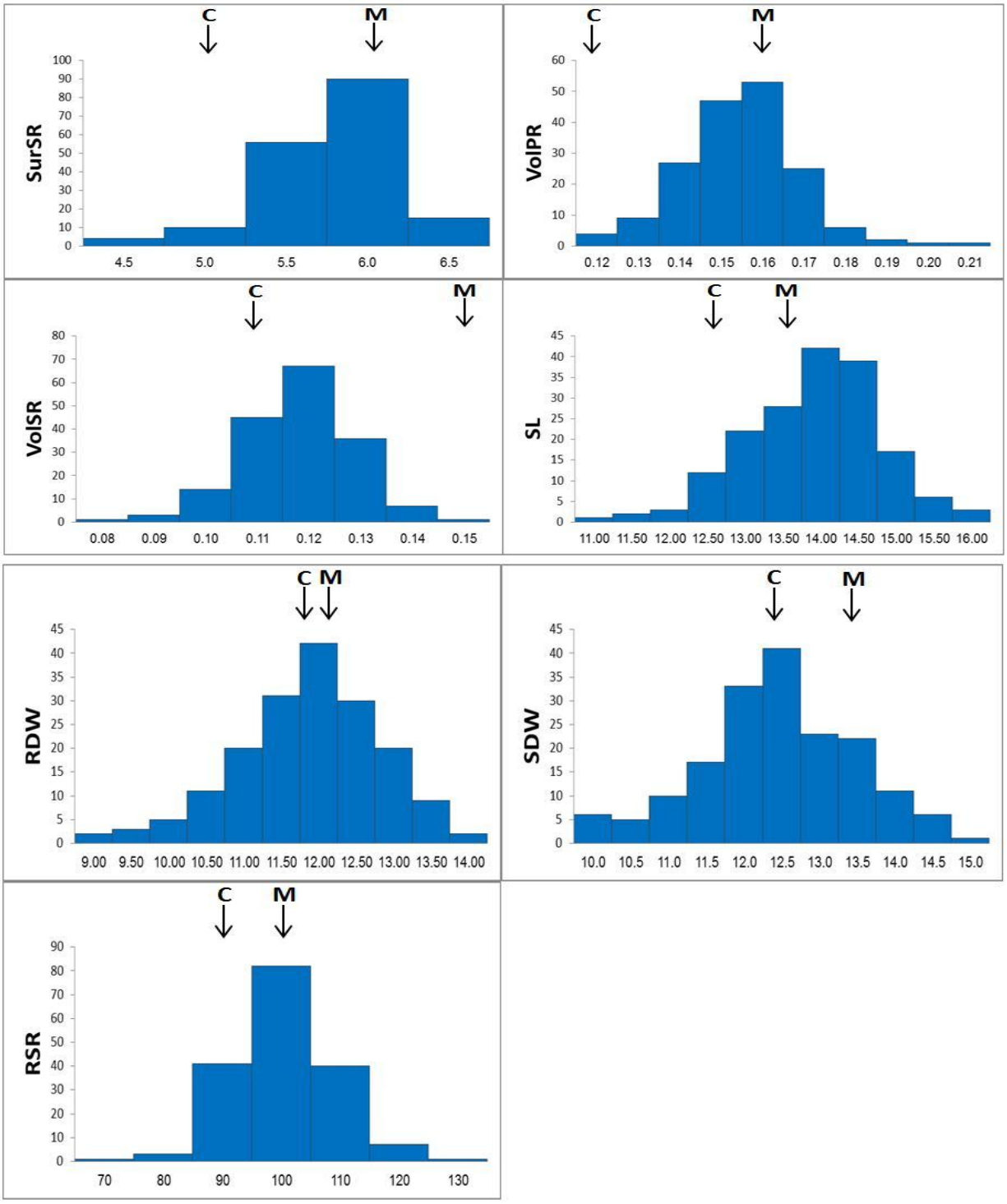

Fig. 2 Continued …

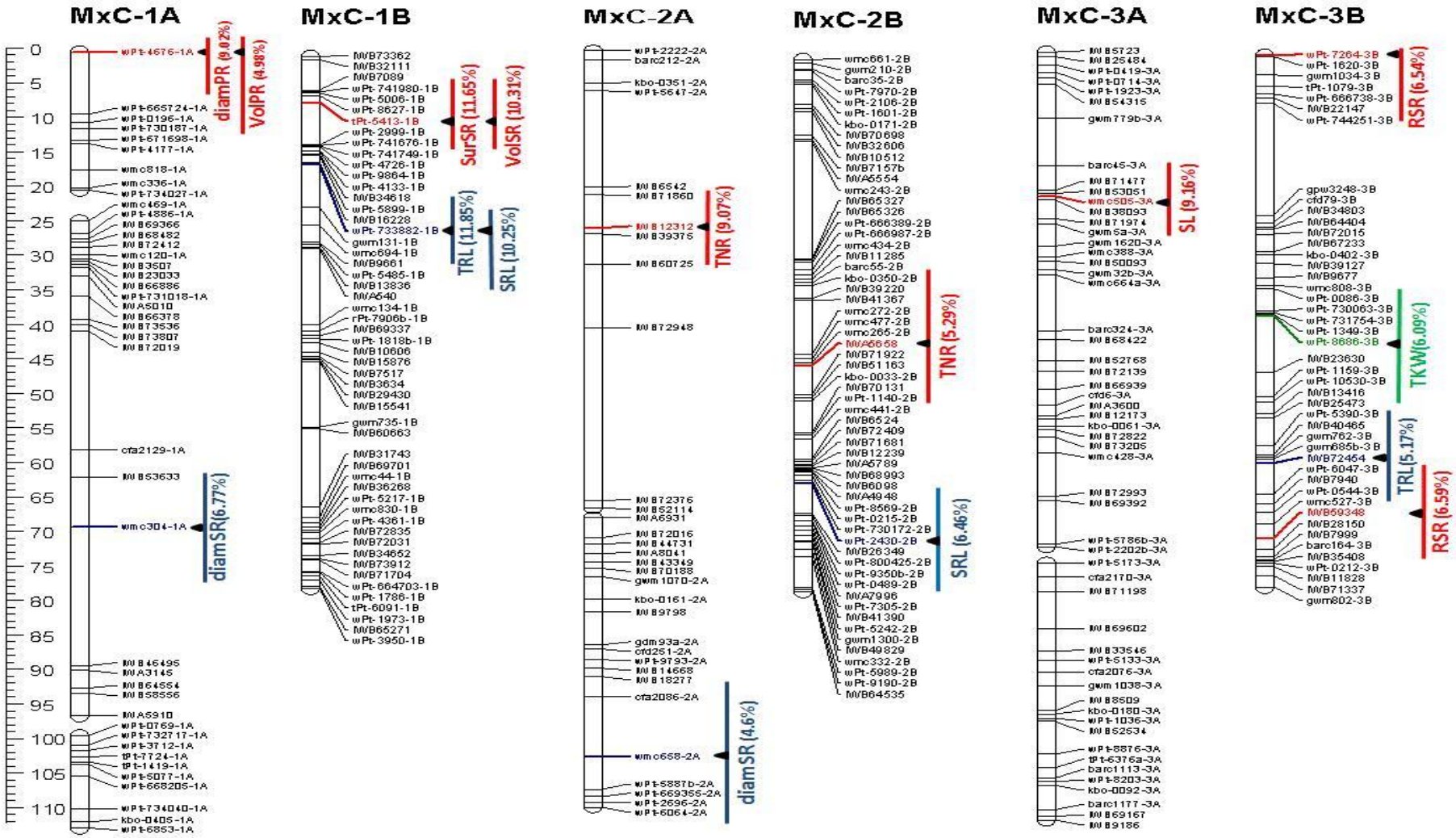

Fig. 3 Linkage map and quantitative trait loci (QTL) for RSA traits in 176 Meridiano x Claudio RILs of durum wheat

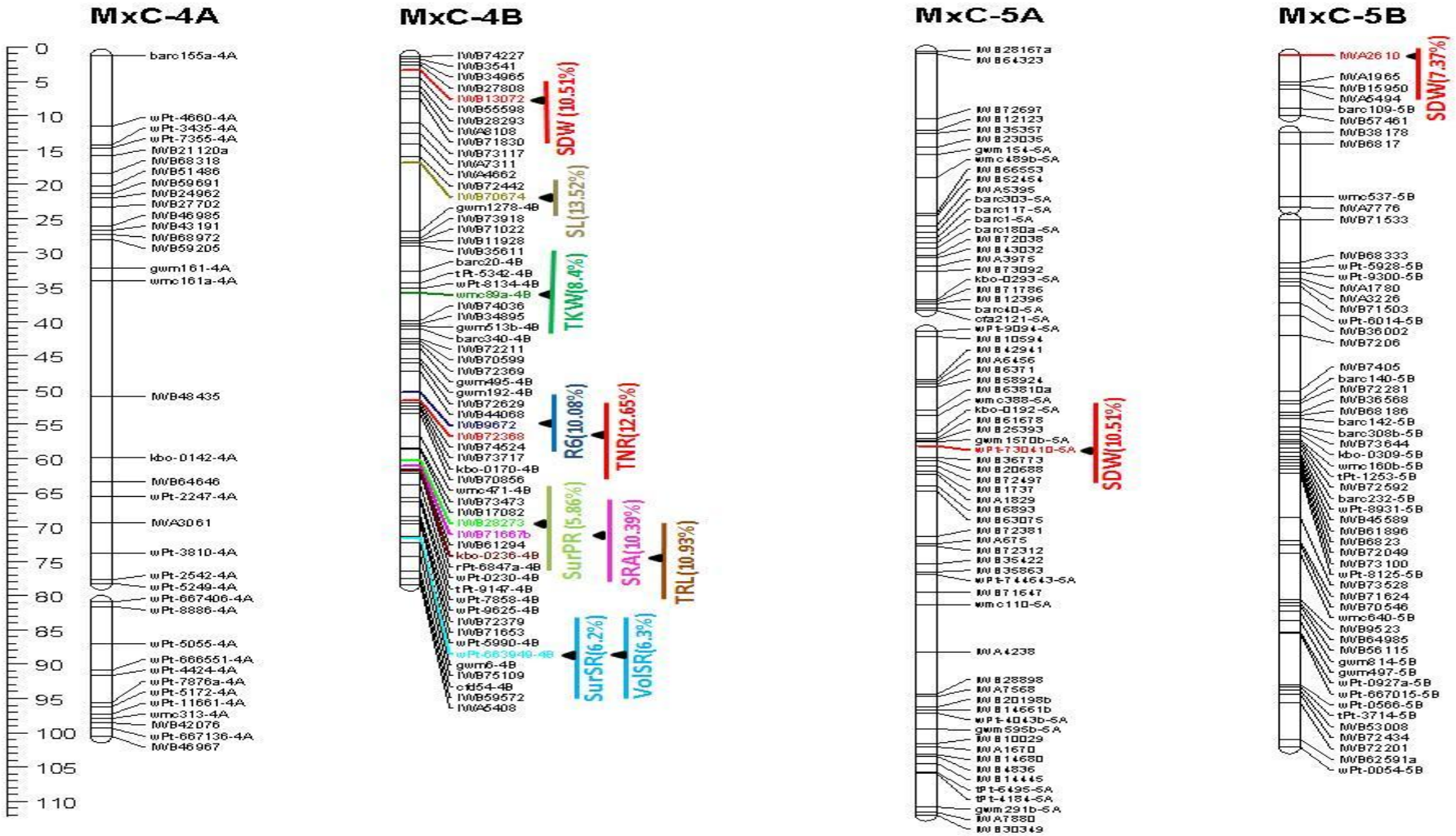

Fig. 3 Continued …

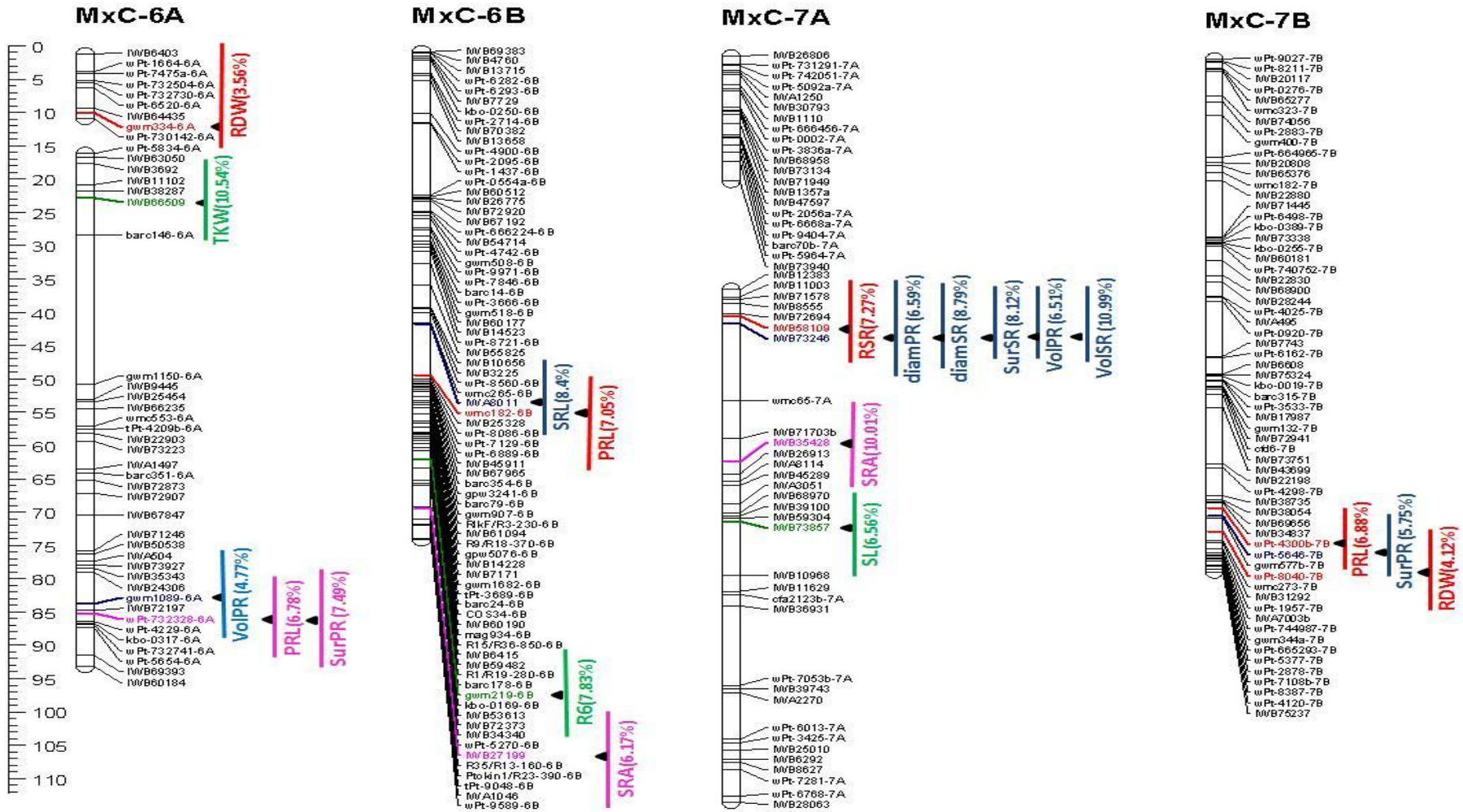

Fig. 3 Continued …

TABLES

Table 1 List of root system architecture traits with explanations and abbreviations.

Trait	**Abbreviation**
Thousand kernel weight	**TKW**
Seminal root angle	**SRA**
Total number root	**TNR**
Percent of 6th root	**R6**
Primery root length	**PRL**
Seminal root length	**SRL**
Total root length	**TRL**
diameter primary root	**diamPR**
diameter Seminal root	**diamSR**
Surface of primary root	**SurPR**
Surface of seminal root	**SurSR**
Volume of primary root	**VolPR**
Volume of seminal root	**VolSR**
Shoot length	**SL**
Root dry weight	**RDW**
Shoot dry weight	**SDW**
Root shoot ratio	**RSR**

Table 2 Summary of traits heritability, coefficient of variation and genetic differences as obtained in the analysis of 176 Meridiano x Claudio RILs at the seedling stage

Trait	TKW	SRA	TNR	R6	PRL	SRL	TRL	diamPR	diamSR	SurPR	SurSR	VolPR	VolSR	SL	RDW	SDW	RSR
Mean	48.3	97.9	5.0	6.0	30.7	22.9	122.5	0.08	0.08	7.5	5.6	0.2	0.11	13.7	11.6	12.2	95.5
Max	63.4	128.5	5.7	58.1	34.5	26.2	142.4	0.10	0.09	9.0	6.3	0.2	0.15	15.6	13.7	14.7	122.4
Min	39.8	68.3	4.4	0.0	24.7	17.7	95.8	0.07	0.07	5.8	4.0	0.1	0.08	10.9	8.6	9.6	69.7
F (P)	**	**	**	**	**	**	**	*	*	ns	ns	ns	ns	**	*	**	**
CV (%)	6.35	13.5	13.4	13.5	5.68	6.56	7.12	5.02	5.22	8.74	9.55	13.31	13.69	7.0	13.8	12.6	13.02
h^2 (%)	78.62	65.35	44.30	64.20	29.70	27.51	30.72	26.29	14.35	16.66	29.62	11.04	15.70	49.06	20.56	29.88	26.30

Comparison among mean values significant at nonsignificant (ns), *P <0.05 and **P < 0.01 (F Test)

Table 3 Correlation coefficients between RSA traits in 176 Meridiano x Claudio RILs at the seedling stage

Trait	TKW	SRA	TNR	R6	PRL	SRL	TRL	diamPR	diamSR	SurPR	SurSR	VolPR	VolSR	SL	RDW	SDW
TKW	-															
SRA	-0.12															
TNR	-0.13	0.12														
R6	-0.14	0.07	0.71**													
PRL	-0.45**	0.04	0.15*	0.20**												
SRL	-0.33**	-0.14	0.07	0.01	0.71**											
TRL	-0.36**	-0.03	0.59**	0.42**	0.75**	0.83**										
diamPR	0.09	0.06	0.14	0.09	-0.01	0.05	0.10									
diamSR	0.13	0.03	0.13	0.09	0.01	0.08	0.12	0.87**								
SurPR	-0.35**	0.07	0.20**	0.22**	0.79**	0.58**	0.64**	0.59**	0.55**							
SurSR	-0.19**	-0.10	0.12	0.07	0.58**	0.83**	0.72**	0.51**	0.60**	0.77**						
VolPR	-0.18*	0.06	0.19*	0.19*	0.50**	0.39**	0.45**	0.84**	0.76**	0.92**	0.74**					
VolSR	0.15*	-0.07	0.08	0.05	0.30**	0.50**	0.43**	0.74**	0.85**	0.69**	0.88**	0.80**				
SL	-0.24**	0.08	0.21**	0.17*	0.36**	0.30**	0.38**	-0.09	-0.05	0.24**	0.22**	0.12	0.08			
RDW	-0.35**	0.06	0.30**	0.12	0.47**	0.51**	0.58**	0.13	0.14*	0.46**	0.48**	0.36**	0.30**	0.39**		
SDW	-0.08	0.03	0.14	0.11	0.30**	0.34**	0.36**	-0.05	-0.03	0.19**	0.26**	0.10	0.15*	0.70**	0.51**	
RSR	-0.26**	0.03	0.15*	0.01	0.15*	0.14	0.19**	0.17*	0.16*	0.25**	0.19**	0.24**	0.13	-0.33**	0.47**	-0.52**

* and ** statistically different from zero at 0.05 and ≤ 0.01 respectively

Table 4 QTL detected for RSA traits in 176 Meridiano x Claudio RILs of durum wheat collected at the seedling stage (with exclusion of TKW)

Trait	QTL	Chromosome	Marker	LOD	Position	Position-CL/2	Position+CL/2	R^2 %	Additive effect*	Parent
TKW	*QTKW1-3B*	3B	wPt-8686-3B	2.82	68	61	76	6.09	-1.08	Claudio
	QTKW2-4B	4B	wmc89a-4B	3.26	60	55	66	8.40	-1.27	Claudio
	QTKW3-6A	6A	IWB66509	4.48	8	4	12	10.54	-1.43	Claudio
SRA	*QSRA1-4B*	4B	IWB71667b	4.41	96	91	100	10.39	-3.23	Claudio
	QSRA2-6B	6B	IWB27199	2.57	191	183	198	6.17	2.50	Meridiano
	QSRA3-7A	7A	IWB35428	3.27	31	26	35	10.01	3.17	Meridiano
TNR	*QTNR1-2A*	2A	IWB12312	3.06	17	12	22	9.07	0.05	Meridiano
	QTNR2-2B	2B	IWA5658	2.54	100	912	109	5.29	0.04	Meridiano
	QTNR3-4B	4B	IWB72368	5.63	81	77	84	12.65	0.06	Meridiano
R6	*QR6-1-4B*	4B	IWB9672	4.24	79	74	83	10.08	3.13	Meridiano
	QR6-2-6B	6B	gwm219-6B	3.42	170	164	176	7.83	2.75	Meridiano
PRL	*QPRL1-6A*	6A	wPt-732328	3	83	76	90	6.78	0.42	Meridiano
	QPRL2-6B	6B	wmc182-6B	2.9	135	129	142	7.05	-0.44	Claudio
	QPRL3-7B	7B	wPt-4300b-7B	2.85	202	195	209	6.88	0.44	Meridiano
SRL	*QSRL1-1B*	1B	wPt-733882-1B	3.69	55	51	60	10.25	-0.42	Claudio
	QSRL1-2B	2B	wPt-2430-2B	2.98	139	132	146	6.46	-0.33	Claudio
	QSRL1-6B	6B	IWA8011	2.88	129	123	134	8.4	-0.38	Claudio
TRL	*QTRL1-1B*	1B	wPt-733882-1B	3.84	50	46	54	11.85	-2.72	Claudio
	QTRL2-3B	3B-1	IWB72454	2.42	108	99	117	5.17	1.79	Meridiano
	QTRL3-4B	4B	kbo-0236-4B	5.16	97	93	101	10.93	2.60	Meridiano
diamPR	*QdiamPR1-1A*	1A	wPt-4676-1A	3.88	0	0	5	9.02	0.01	Meridiano
	QdiamPR2-7A	7A	IWB73246	2.56	7	0	14	6.59	0.01	Meridiano
diamSR	*QdiamSR1-1A*	1A	wmc336-1A	3.05	18	11	25	6.77	0.01	Meridiano
	QdiamSR2-2A	2A	wmc658-2A	2.09	23	12	33	4.6	0.01	Meridiano
	QdiamSR3-7A	7A	IWB73246	3.22	8	2	13	8.79	0.01	Meridiano

*Additive effects, Calculated as half of the difference between the mean value of the RILs homozygous for the Claudio allele and the mean value of the RILs homozygous for the Meridiano allele. Allelic effects positive in sign indicate that the allele increasing the trait originates from Meridiano

Table 4 Continued …

Trait	QTL	Chromosome	Marker	LOD	Position	Position-CL/2	Position+CL/2	R^2 %	Additive effect	Parent
SurPR	*QSurPR1-4B*	4B	IWB28273	2.4	95	87	103	5.86	0.18	Meridiano
	QSurPR2-6A	6A	wPt-732328-6A	3.32	83	77	89	7.49	0.14	Meridiano
	QSurPR3-7B	7B	wPt-5646-7B	2.46	204	195	212	5.75	0.12	Meridiano
SurSR	*QSurSR1-1B*	1B	tPt-5413-1B	5.01	18	14	22	11.65	-0.13	Claudio
	QSurSR2-4B	4B	wPt-663949-4B	3.15	113	106	120	6.82	0.10	Meridiano
	QSurSR3-7A	7A	IWB73246	3.5	6	0	12	8.12	0.11	Meridiano
VolPR	*QVolPR1-1A*	1A	wPt-4676-1A	2.19	0	0	9	4.98	0.01	Meridiano
	QVolPR2-6A	6A	gwm1089-6A	2.12	81	72	91	4.77	0.01	Meridiano
	QVolPR3-7A	7A	IWB73246	2.73	6	0	13	6.51	0.01	Meridiano
VolSR	*QVolSR1-1B*	1B	tPt-5413-1B	4.32	18	13	22	10.31	-0.01	Claudio
	QVolSR2-4B	4B	wPt-663949-4B	2.94	113	105	120	6.30	0.01	Meridiano
	QVolSR3-7A	7A	IWB73246	4.36	7	3	11	10.99	0.01	Meridiano
SL	*QSL1-3A*	3A	wmc505-3A	4.08	24	19	29	9.16	-0.27	Claudio
	QSL2-4B	4B	IWB70674	4.17	25	22	28	13.52	0.32	Meridiano
	QSL3-7A	7A	IWB73857	2.51	47	40	54	6.56	-0.22	Claudio
RDW	*QRDW1-6A*	6A	gwm334-6A	2.47	11	0	24	3.56	-0.18	Claudio
	QRDW2-7B	7B	wPt-8040-7B	2.64	211	199	222	4.12	0.20	Meridiano
SDW	*QSDW1-4B*	4B	IWB13072	2.98	3	0	8	10.51	0.34	Meridiano
	QSDW2-5A	5A	wPt-730410-5A	3.05	32	26	39	7.37	-0.28	Claudio
	QSDW3-5B	5B	IWA2610	2.46	0	0	6	7.48	0.28	Meridiano
RSR	*QRSR1-3B*	3B	wPt-7264-3B	3.08	0	0	7	6.54	2.07	Meridiano
	QRSR2-3B	3B	IWB59348	2.78	127	119	134	6.59	2.08	Meridiano
	QRSR3-7A	7A	IWB58109	3.11	4	0	11	7.27	2.19	Meridiano

CHAPTER 5. GENETIC VARIATION FOR AERENCHYMA AND OTHER ROOT ANATOMICAL TRAITS IN DURUM WHEAT

5.1 ABSTRACT

Variation in root anatomical traits influences whole plant physiology and crop adaptation to adverse soil conditions and thus impacts yield and its stability. Typical components of anatomical root traits are the arrangement of cells and tissues as observed by microscopy sections. In this study, we investigated the phenotypic variation of eleven root anatomical traits including aerenchyma features in ten elite durum wheat cultivars and found significant differences among cultivars for several traits. Trait heritability ranged from 0.12 (number of xylem vessels) to 0.72 (number of aerenchyma lacunae). While area and number of aerenchyma lacunae were highly correlated, neither trait correlated with other root features, suggesting an independent physiological and/or genetic control in respect to the other root anatomical traits. The old Italian founder cultivar Cappelli was shown to have a significantly higher portion of root aerenchyma of all the modern cultivars. These results show for the first time the presence of sizeable genetic variation in aerenchyma-related root anatomical traits in cultivated tetraploid wheats, prompting for additional studies aimed at mapping the quantitative trait loci governing such variation and to test their role in the adaptive response of durum wheat to abiotic stresses as related to soil conditions.

Key words: Aerenchyma; Anatomical root traits; Durum wheat; Root stele; *Triticum turgidum*

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5.2 INTRODUCTION

Among cereals, durum wheat (*Triticum turgidum* L. var. *durum*, 2n = 4x = 28; AABB genomes) is one of the oldest cultivated species and is an important crop for Mediterranean countries where more than half of the global acreage of this crop is grown (Key 2005; Maccaferri et al. 2008; Shewry 2009).

Traditionally, efforts in plant breeding have focused on shoot characteristics rather than on roots, owing in part to the challenges of *in situ* examination of roots and the complex influence of the environment on their growth (Tuberosa et al. 2002). Roots play several essential roles in the plant life cycle, including anchoring to the soil, mechanical support to stems, uptake of water and nutrients (Osmont et al. 2007; Smith and De Smet 2012) and, more in general, governing the adaptive response of the plant to unfavourable soil conditions (De Dorlodot et al. 2007; Reynolds and Tuberosa 2008). In view of this and the continuous improvement in high-throughput phenotyping, root system architecture is receiving increasing attention in terms of genetic dissection and physiology (Gregory et al. 2009; Trachsel et al. 2011; Lobet et al. 2014) including small grain cereals (Habash et al. 2009; Naz et al. 2014; Cané et al. 2014; Petrarulo et al. 2014).

Much less is known about genetic variation of internal anatomical structures of roots, including the arrangement of cells (number, dimension and orientation) and tissues (features of cortex, stele, etc.), and their developmental dynamics and physiology (Burton et al. 2013). This notwithstanding, a number of studies have already linked root anatomy with adaptation and tolerance to abiotic stress (Wahl and Ryser, 2000; Setter and Water 2003; Gowda et al. 2011; Lynch 2013). The root cortex is thought to play an important role in storage and transport, based on its considerable vacuolar space and the collective absorptive surface area of its cells (Lopez Bucio et al. 2003). It has been suggested that large cortical cell size improves drought tolerance in maize by reducing root metabolic costs (Chimungo et al. 2014) and that xylem vessels features affect axial water conductance (Lynch et al. 2014). Aerenchyma, i.e. the presence of empty spaces in the roots has been linked to increased tolerance to waterlogging in barley, maize, rice and wheat (Thomson et al. 1990; Setter and Waters, 2003; Saqib et al. 2005; Yamauchi et al. 2013). Aerenchyma has also been associated with deeper rooting under drought and increased drought tolerance in maize by reducing metabolic cost (Zhu et al. 2010; Lynch et al. 2014), although it may inhibit radial nutrient transport (Hu et al. 2014). In

summary, root anatomy appears to play a major role in root physiology, eventually influencing crop performance particularly under adverse environmental conditions.

We report the results of a phenotypic screening for anatomical root traits in durum wheat cultivars with emphasis on root aerenchyma. To our best knowledge, this is the first investigation of this type in durum wheat. The presence of genetic variation for root anatomical traits and the possibility to rapidly phenotype them could provide novel opportunities for durum wheat breeding programs aiming to enhance yield potential and yield stability.

5.3 MATERIALS AND METHODS

Ten durum wheat cultivars (Cappelli, Claudio, Colosseo, Levante, Lloyd, Meridiano, Normanno, Rascon/2*Tarro, Saragolla and Simeto) were utilized in this study. Pedigrees and additional information are reported in Table 1. These ten cultivars well represent the genetic diversity present in the cultivated durum wheat gene pool, as described elsewhere (Maccaferri et al. 2005).

Root samples were collected from plants at early stem-elongation phase grown in an openfield experiment (in Cadriano, near Bologna, Italy) in 2-m-long plots with eight rows 0.15 m apart and 0.70 m between each double row, at a density of 400 seeds m-2, in a randomized complete block design with two replications. Two representative plants per plot and two roots per plant were utilized for microscopical sections. Attention was given in sampling nodal (i.e. crown) roots, at the base of the 2nd whorl. Root sections were always taken at 2 cm from the insertion to the crown. Sections were visualized using a Nikon Eclipse 50i microscope integrated with a Video Camera Module (Nikon, DS Camera Head DS-5M). A camera control unit (DS camera control unit DA-L1) was used to capture and save the images. Analysis of root digital images was performed with the program RootScan (Burton et al. 2012a). Based on the analysis of digital images, eleven anatomical root traits were considered (Table 2). The following traits were measured: area of aerenchyma (AA), cortex cell wall area (CCWA), root cross-sectional area (RXSA), stele cell wall area (SCWA), total cortical area (TCA) and cross-sectional cell wall area (XSCWA). Traits values were calibrated from pixels using an image of a 1 mm micrometer taken at the same magnification as the analyzed images (1 linear mm = 295 pixels). The area of cells in the cortex (CCA) was obtained as secondary measurements as follows: CCA = cortical area - aerenchyma area. Count-based traits included count of cortex cell (CCC), number of aerenchyma lacunae (NAL), number of cell files in the cortex

(NCF) and number of xylem vessels (NXV). Xylem vessels were distinguished from other objects by using the maximum area difference in a ranked list of stele object areas, so that objects with areas above the value of maximum difference are defined as xylem, after manual exclusion of large obviously erroneous objects that appeared in the stele center. A visual summary of the trait collection process is provided in Fig. 1. Analysis of variance (ANOVA) was performed using the software Genstat v.

15. Means comparisons were conducted using Tukey's test ($P \leq 0.05$). Genotypic and phenotypic variances were estimated using expected mean squares and then used to calculate heritability (h^2) according to the following formula:

$$h^2 = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_E}{r}}$$

where σ^2_G and σ^2_E represent the genotypic and the environmental components of the phenotypic variance, respectively, and r the number of replications. Heritability should be considered as being 'narrow sense' because the genetic variance included only the additive component and, possibly, the additive × additive epistatic interaction (Sanguineti, et al. 2007).

5.4 RESULTS AND DISCUSSION

ANOVA identified significant differences among the durum wheat cultivars for aerenchyma area (AA), cortex cell wall area (CCWA), number of aerenchyma lacunae (NAL), root cross section area (RXSA), stele cell wall area (SCWA), total cortex area (TCA) and cell wall cross section area (XSCWA). No significant differences among cultivars were detected for cortex cells area (CCA), count of cortex cells (CCC), number of cell files in cortex (NCF) and number of xylem vessels (NXV) (Table 3). Example images of root cross sections are provided in Fig. 2.

AA and NA, the two traits related with aerenchyma, showed the highest h^2 values, ranging from 67 to 72%, respectively (Table 3). The Italian cv. Cappelli, an old durum wheat founder (released in 1915) always showed the highest values for aerenchyma-related traits and was found to be highly significantly different from all other cvs. (Fig. 3). The only other cvs. showing an appreciable presence of aerenchyma were Levante and Lloyd.

For traits related with root cross-section area (CCWA, RXSA, SCWA, TCA and XSCWA) intermediate h^2 values (56, 46, 69, 62 and 52%, respectively; Table 3) were observed. For these traits, cvs. Lloyd and Simeto always showed the highest values (Fig. 3).

Correlation analysis (Table 4) showed highly significant correlations among CCA, CCWA, RXSA, SCWA, TCA and XSCWA ($r > 0.85$; $P < 0.01$), i.e. all traits related to areas of root sections, including both cortex and stele regions. Notwithstanding the highly significant, positive correlation ($r = 0.86$; $P < 0.01$) between number of cortical cells (CCC) and cell files (NCF), cvs. did not differ for these traits and CCC and NCF were not correlated with any other traits. A high, positive correlation ($r = 0.95$; $P < 0.01$) was observed between AA and NAL. These two traits did not correlate with any of the other traits, suggesting a different physiological and/or genetic control as compared to the other traits. In maize, aerenchyma was shown to be under allometric scaling (i.e. correlation with total plant size or biomass; Burton et al. 2013). However, although a similar correlation cannot be excluded in durum wheat, it is unlikely to have been of any relevance in our experiment since plants were sampled at early stem elongation, with hardly any difference in plant size.

In cereals, aerenchyma forms in the root cortex by programmed death of parenchyma cells, resulting in replacement of cells with air-filled channels called lacunae (Burton et al. 2012b).

Aerenchyma was previously shown to be an adaptive mechanism adopted by plant roots in response to stress events (most often waterlogging and drought; Setter and Waters 2003), and/or a constitutive, genetically controlled feature (i.e. not triggered by stress. Yamauchi et al. 2013).

Genetically controlled differences in root aerenchyma were observed in *Hordeum* (Garthwaite et al. 2003) and *Trypsacum* (Ray et al. 1998) and more extensive investigations allowed for the mapping of aerenchyma quantitative trait loci (QTLs) in maize (Mano et al. 2012; Burton et al. 2013, 2015; Hu et al. 2014) and rice (Nionesa et al. 2013). Aerenchyma was also analyzed in a *Lotus japonicus* RIL population, although no QTL was identified (Striker et al. 2014).

In maize, aerenchyma was considered in the definition of new root ideotypes because of possible effects on root metabolic demand and radial transport of nutrients, both expected to be lower in presence of abundant root cortical aerenchyma (Jaramillo et al. 2013; Lynch 2013; Hu et al. 2014). Accordingly, high aerenchyma formation in maize was correlated with reduced root respiration,

increased rooting depth, improved leaf water status, increased plant biomass and substantially improved yield under drought conditions (Zhu et al. 2010).

It is intriguing that the old durum wheat cv. Cappelli, a direct selection from a landrace ('Jennah Khetifa'. Laidò et al. 2013) originating from a North African drought-prone environment showed the strongest difference in root anatomy as far as aerenchyma is concerned. Cappelli had already shown relatively high water-use efficiency (Rizza et al. 2012). Based on these observations, it can be hypothesized that Cappelli's relatively extreme aerenchyma phenotype is the result of the adaptation of the original landrace to dry and/or low-fertility growing conditions. In these conditions, root growth and rooting depth could be sustained in aerenchyma-rich roots thanks to their reduced metabolic cost (Lynch et al. 2014). This hypothesis is also in line with previous observations that have recognized the considerable potential of exotic germplasm to provide traits for drought-adaptive mechanisms in wheat (Reynolds et al. 2007).

5.5 CONCLUSIONS

Variation for anatomical root traits, i.e the ontogenetic arrangement of root cells and tissues, has been correlated with crop performance in terms of both yield potential and stability (Lynch et al. 2014). Here, we showed for the first time the presence of highly significant genetic differences among durum wheat cultivars for area and number of aerenchyma lacunae, which are traits previously shown to be associated to crop performance in response to abiotic stress, and for additional traits related with root anatomy. Notably, among the ten cvs. considered in our study, the old Italian founder Cappelli, derived from a North African landrace, was shown to be particularly rich in aerenchyma, while only two other cvs. showed a modest presence of aerenchyma. The evaluation of mapping populations developed starting from the cross of Cappelli with other cvs. herein investigated will provide a means to identify the QTLs involved in aerenchyma formation in durum wheat while assessing their role in regulating the adaptive response of the crop to an excess or lack of water in the soil.

5.6 REFERENCES

Burton AL, Lynch JP, Brown KM (2012a) RootScan: software for high-throughput analysis of root anatomical traits. Plant Soil 357:189-203

Burton AL, Lynch JP, Brown KM (2012b) Spatial distribution and phenotypic variation in root cortical aerenchyma of maize (Zea mays L.) Plant Soil 357:1453-1457

Burton AL, Brown KM, Lynch JP (2013) Phenotypic diversity of root anatomical and architectural traits in Zea species. Crop Sci 53:1042-1055

Burton AL, Johnson J, Foerster J, Hanlon MT, Kaeppler SM, Lynch JP, Brown KM (2015) QTL mapping and phenotypic variation of root anatomical traits in maize (Zea mays L.). Theor Appl Genet 128:93-106

Canè MA, Maccaferri M, Nazemi G, Salvi S, Francia R, Colalongo C, Tuberosa R (2014) Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. Mol Breed 34:1629-1645

Chimungu JG, Brown KM, Lynch JP (2014) Large root cortical cell size improves drought tolerance in maize. Plant Physiol 166:2166-2178

De Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci 12: 474-481

Garthwaite AJ, von Bothmer R, Colmer TD (2003) Diversity in root aeration traits associated with waterlogging tolerance in the genus Hordeum. Funct Plant Biol 30:875-889

Gowda VRP, Henry A, Yamauchi A, Shashidhar HE, Serraj R (2011) Root biology and genetic improvement for drought avoidance in rice. Field Crops Research 122:1-13

Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young IM (2009) Root phenomics of crops: opportunities and challenges. Funct Plant Biol 36:922-929

Habash DZ, Kehel Z, Nachit M (2009) Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J Exp Bot 60:2805-2815

Hu B, Henry A, Brown KM, Lynch JP (2014) Root cortical aerenchyma inhibits radial nutrient transport in maize (Zea mays). Ann Bot 113:181-189

Jaramillo RE, Nord EA, Chimungu JG, Brown KM, Lynch JP (2013) Root cortical burden influences drought tolerance in maize. Ann Bot 112:429-437

Key JM (2005) Wheat: its concept, evolution, and taxonomy. In: Royo C, Nachit MM, Di Fonzo N,

Araus JL, Pfeiffer WH, Slafer GA (eds.) Durum wheat breeding: current approaches and future strategies, Vol. 1. New York, London, Oxford: Food Products Press, an imprint of the Haworth Press Inc., pp 3-61

Laidò G, Mangini G, Taranto F, Gadaleta A, Blanco A, Cattivelli L, Marone D, Mastrangelo AM, Papa R, De Vita P (2013) Genetic diversity and population structure of tetraploid wheats (Triticum turgidum L.) estimated by SSR, DART and pedigree data. PLoS One 8:e67280

Lobet G, Couvreur V, Meunier F, Javaux M, Draye X (2014) Plant water uptake in drying soils. Plant Physiol 164:1619-1627

Lopez-Bucio J, Cruz-Ramirez A, Herrera-Estrella L, 2003. The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6: 280-287

Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347-357

Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. J Exp Bot 65:6155-6166

Maccaferri M, Sanguineti MC, Noli E, Tuberosa R (2005) Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Mol Breed 15:271-289

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Ben Salem M, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178:489-511

Mano Y, Omori F, & Takeda K (2012) Construction of intraspecific linkage maps, detection of a chromosome inversion, and mapping of QTL for constitutive root aerenchyma formation in the teosinte Zea nicaraguensis. Mol Breed 29:137-146

Naz A, Arifuzzaman M, Muzammil S, Pillen K, Léon J (2014) Wild barley introgression lines revealed novel QTL alleles for root and related shoot traits in the cultivated barley (Hordeum vulgare L.). BMC Genet 15:107

Niones JM, Suralta R, Inukai Y, & Yamauchi A (2013) Roles of root aerenchyma development and its associated QTL in dry matter production under transient moisture stress in rice. Plant Prod Sci 16:205-216

Osmont KS, Sibout R, Hardtke CS (2007) Hidden branches: developments in root system architecture. Annu Rev Plant Biol 58:93-113

Petrarulo M, Marone D, Ferragonio P, Cattivelli L, Rubiales D, De Vita P, Mastrangelo AM (2014) Genetic analysis of root morphological traits in wheat. Mol Genet Genomics. doi:10.1007/s00438-014-0957-7

Ray JD, Kindiger B, Dewald CL, Sinclair TR (1998) Preliminary survey of root aerenchyma in Tripsacum. Maydica 43:49-53

Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. Curr Op Plant Biol 11:171-179

Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. J Exp Bot 58:177-186

Rizza F, Ghashghaie J, Meyer S, Matteu L, Mastrangelo AM, Badeck F-W (2012) Constitutive differences in water use efficiency between two durum wheat cultivars. Field Crop Res 125:49-60

Sanguineti MC, Li S, Maccaferri M, Cornet S, Rotondo F, Chiari T, Tuberosa R (2007) Genetic dissection of seminal root architecture in elite durum wheat germplasm. Ann Appl Biol 151: 291-305

Saqib M, Akhtar J, Qureshi RH (2005) Na^+ exclusion and salt resistance of wheat (Triticum aestivum) in saline-waterlogged conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. Plant Sci 169:125-130

Setter TL, Waters I (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. Plant Soil 253:1–34

Shewry PR (2009) Wheat. J Exp Bot 60:1537–1553

Smith S, De Smet I (2012) Root system architecture: insights from Arabidopsis and cereal crops. Philos Trans R Soc Lond B Biol Sci 367:1441-1452

Striker GG, Casas C, Manzur ME, Ploschuk RA, Casal JJ (2014) Phenomic networks reveal largely independent root and shoot adjustment in waterlogged plants of Lotus japonicus. Plant Cell Environ 37:2278-2293

Thomson CJ, Armstrong W, Waters I, Greenway H (1990) Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. Plant Cell Environ 13:395-403.

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011) Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. Plant Soil 341:75-78

Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Mol Biol 48:697-712

Wahl S, Ryser P (2000) Root tissue structure is linked to ecological strategies of grasses. New Phytol 148:459-471

Yamauchi T, Shimamura S, Nakazono M, & Mochizuki T (2013) Aerenchyma formation in crop species: a review. Field Crops Res 152:8-16

Zhu JM, Brown KM, Lynch JP (2010) Root cortical aerenchyma improves the drought tolerance of maize (Zea mays L.). Plant Cell Environ 33:740-

FIGURES

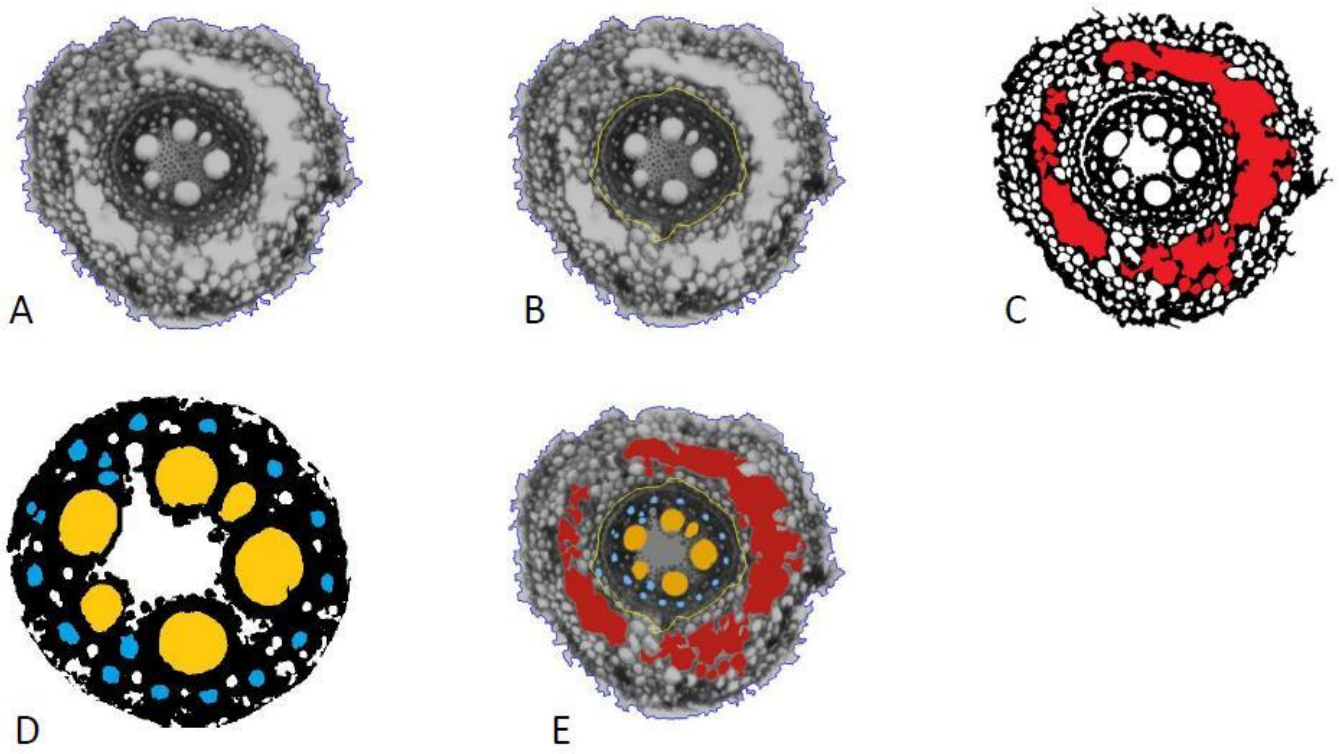

Fig. 1 Description of the main root anatomical features collected in this study using the software Rootscan. A) The whole root cross section is isolated from the background (blue line). B) The stele is separated from the cortex (yellow line). C) The aerenchyma is identified (in red). D) Within the stele portion, the xylem (in yellow) and protoxylem vessels (in blue) are identified. E) Final elaboration with all anatomical parts highlighted

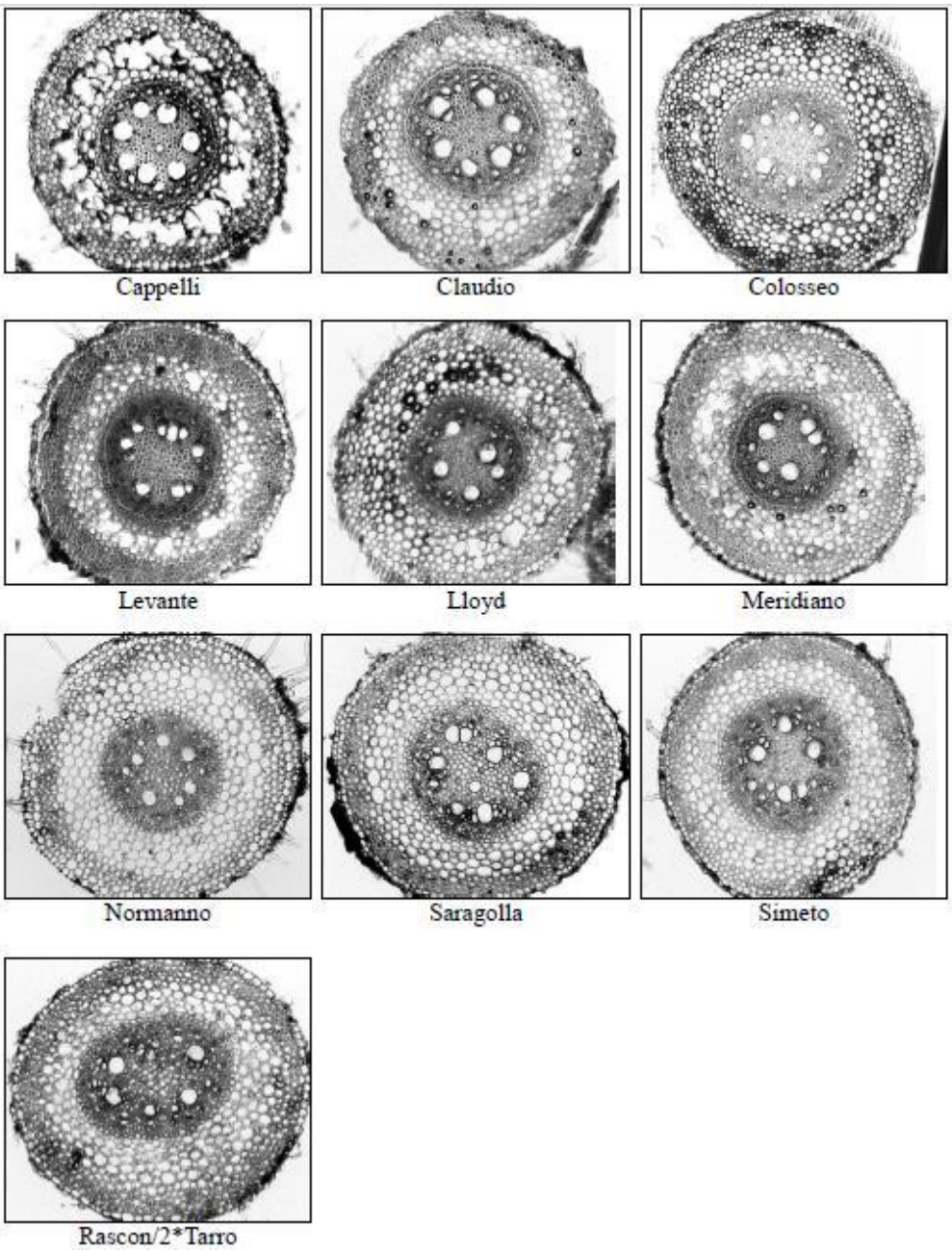

Fig. 2 Sample images of root cross sections of the durum wheat cultivars collected in this study

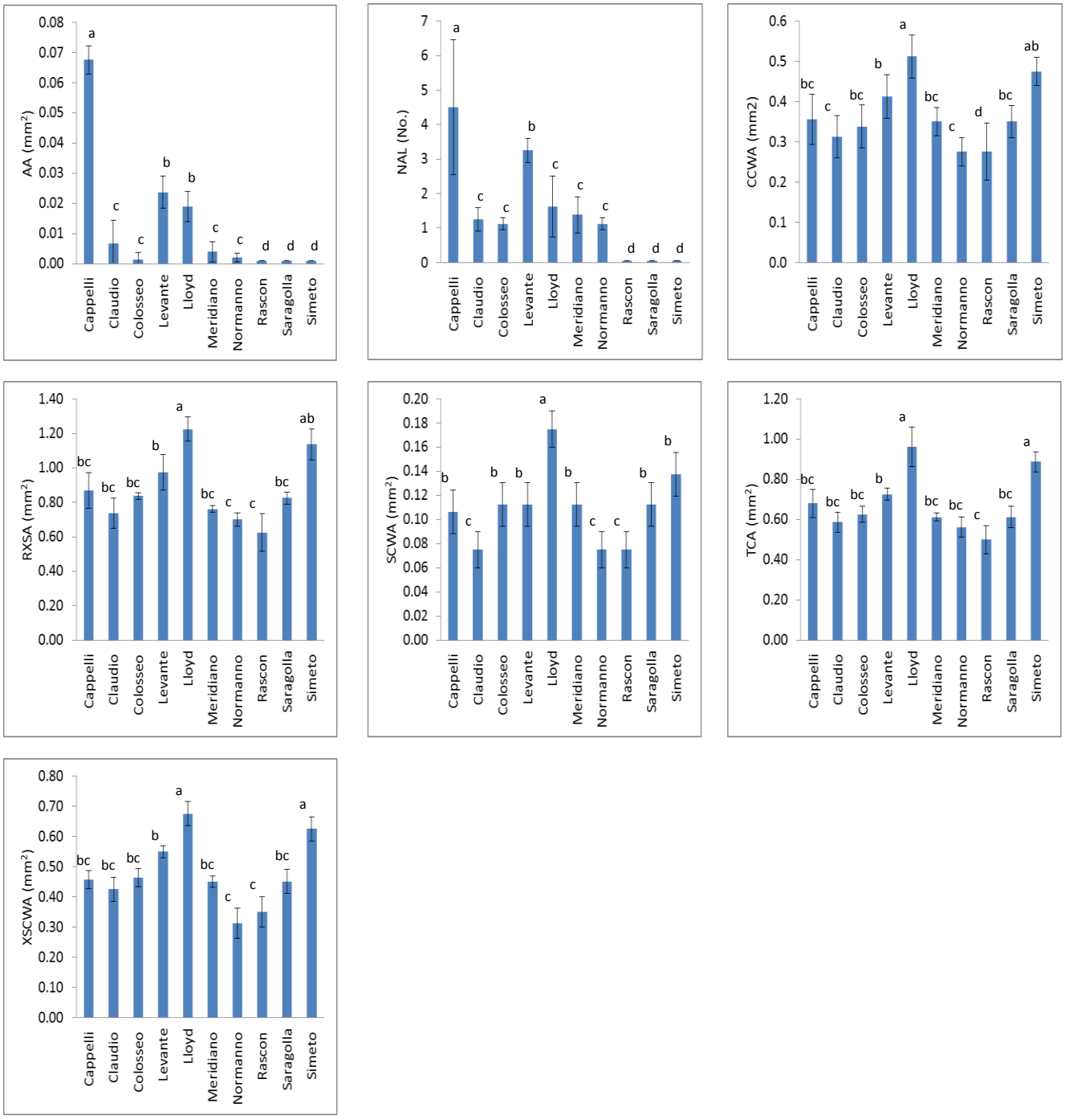

Fig. 3 Mean values of area of aerenchyma (AA), number of aerenchyma lacunae (NAL), cortex cell wall area (CCWA), root cross-sectional area (RXSA), stele cell wall area (SCWA), total cortical

area (TCA) and cross-sectional cell wall area (XSCWA). Bars represent standard deviations. Letters report statistically different comparisons based on Tukey's test ($P < 0.05$)

TABLES

Table 1 Details about the durum wheat cultivars utilized in this study

Cultivar	Registration		Pedigree
	Country	Year	
Cappelli	Italy	1930	selection (by Strampelli) from Jennah Khetifa
Claudio	Italy	1998	CIMMYT selection/Durango//ISI938/Grazia
Colosseo	Italy	1995	Mexa's mutant/Creso
Levante	Italy	2002	G80/Picemo//10M10
Lloyd	USA	1983	Cando/Edmore
Meridiano	Italy	1999	Simeto/WB881//Duilio/F21
Normanno	Italy	2002	Simeto/F22//L35
Rascon /*2Tarro	Mexico	-	Rascon-37/2*Tarro
Saragolla	Italy	2004	Iride/0114
Simeto	Italy	1988	Capeiti 8/Valnova

Table 2 Anatomical root traits investigated in this study and corresponding acronyms

Acronym	Trait	Unit
AA	Area of aerenchyma	mm^2
CCA	Area of cells in the cortex	mm^2
CCC	Count of cortex cells	no.
CCWA	Cortex cell wall area	mm^2
NAL	Number of aerenchyma lacunae	no.
NCF	Number of cell files in the cortex	no.
NXV	Number of xylem vessels	no.
RXSA	Root cross-sectional area	mm^2
SCWA	Stele cell wall area	mm^2
TCA	Total cortical area	mm^2
XSCWA	Cross-sectional cell wall area	mm^2

Table 3 Results of ANOVA and heritability for root anatomical traits investigated in this study

Trait	AA	CCA	CCC	CCWA	NAL	NCF	NXV	RXSA	SCWA	TCA	XSCWA
h^2	0.67	0.31	0.45	0.56	0.72	0.57	0.12	0.46	0.69	0.52	0.62
F (P)	**	ns	Ns	*	**	ns	ns	*	*	*	*
Min	0	0.10	281.2	0.23	0	2.50	4.50	0.55	0.05	0.45	0.25
Max	0.068	0.25	741.2	0.55	6	22.75	8.75	1.28	0.18	0.98	0.70
Mean	0.012	0.16	507.1	0.37	1.73	11.15	6.61	0.87	0.11	0.68	0.48
HSD [1]	0.025	-	-	0.08	0.90	-	-	0.19	0.03	0.15	0.10

ns, * and **: comparison among mean values not significant , significant at $P < 0.05$ and significant at $P < 0.01$ (F Test), respectively

[1] Tukey's Honest Significant Difference

Table 4 Correlation coefficients (r) among root anatomical traits

Trait	CCA	CCWA	NCF	CCC	AA	NAL	RXSA	SCWA	TCA	XSCWA
CCWA	0.87**									
NCF	0.02	-0.30								
CCC	0.32	-0.02	0.86**							
AA	-0.02	0.18	0.09	0.13						
NAL	-0.07	0.15	0.12	0.15	0.95**					
RXSA	0.86**	0.99**	-0.29	-0.05	0.21	0.18				
SCWA	0.93**	0.94**	-0.06	0.16	0.14	0.08	0.92**			
TCA	0.85**	0.98**	-0.33	-0.10	0.21	0.16	0.99**	0.91**		
XSCWA	0.89**	0.99**	-0.30	0.03	0.16	0.15	0.97**	0.92**	0.95**	
NXV	0.52	0.78**	-0.25	-0.06	0.45	0.47	0.72*	0.75*	0.70*	0.76*

* and ** statistically different from zero at $P \leq 0.05$ and 0.01, respectively

CHAPTER 6. MAPPING QUANTITATIVE TRAIT LOCI FOR RESPONSE TO HEAT STRESS USING CELL MEMBRANE STABILITY (CMS) IN A COLLECTION OF DURUM WHEAT

6.1 ABSTRACT

Heat stress due to increased temperature is an agricultural problem in many areas of the world. In this study, the association mapping (AM) strategy based on a panel of 183 elite of durum wheat accessions (Maccaferri et al., 2006) was deployed in order to dissect the genetic control and identify QTLs for response to heat stress. The experiment was conducted using a randomized complete block design with two replications in greenhouse environmental conditions. Cell membrane stability (CMS) was recorded as a proxy index to evaluate the response to heat stress in a three-step experiment: constitutive heat stress response, acquired heat stress response and constitutive-acquired heat stress response. Significant differences among genotypes were observed for all measured CMS traits. The highest heritability ($h^2 = 0.86$) was recorded for constitutive-acquired heat stress response. The panel was profiled with simple sequence repeat, Diversity Arrays Technology and sequence-tagged site markers (957 markers in total). Thirty four single marker/QTL regions were located in all chromosomes; four major QTLs (LOD $\geq$ 3) for constitutive heat stress response were detected on chromosome 5A, 6A, 7B, while one QTL for constitutive-acquired heat stress response was detected on chromosome 6B. The wide range of genetic variation and the limited influence of population structure support the reliability of our results and prompt for additional finer investigations of the physiological bases underlying these QTLs, towards their exploitation in breeding.

Key words: Heat stress response, Cell membrane stability, Durum wheat

6.2 INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. *durum*, 2n = 4x = 28) is one of the most important crop for the agriculture and economy of Mediterranean countries where durum is cultivated on more than half of the available acreage, sharing the cultivation with other small grain cereals as bread wheat and barley (Marti and Slafer 2014). The Mediterranean basin climate is characterized by warm to hot dry summers strongly affecting yield and performance of winter-cereals. Drought and high-temperature often occurs simultaneously in the terminal part of the growing season when can hardly impairs mid or late reproductive stages, including grain filling (Belaid 2000; Morancho 2000; Habash et al. 2009).

In particular, heat-stress adversely affects wheat production in many regions of the world and is particularly detrimental during reproductive development. Heat-stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. Short-term heat stress (acute heat stress) provokes heat shock response, in contrast, long-term heat exposure (chronic heat stress) induces larger scale adaptations. Yield penalties are associated with high temperatures for an extended period of time (mean temperature of the growth cycle being 18°C–25°C, and maximum day temperatures even more than 35°C during grain filling). Moreover, waves of high temperature, usually 10–15 °C above optimal, is considered as heat shock stress and may cause serious reductions in wheat performances (Wahid et al. 2007).

So far, various conventional breeding-based attempts have been adopted to develop heat-tolerant cultivars (Lobell et al. 2012; Pradhan et al. 2012; Gourdji et al. 2013; Deryng et al. 2014). Furthermore, the screening of genetic diversity (both elite and landraces collections), coupled with the recent development of sophisticated molecular tools, has provided additional possibilities to breeders to improve crops for heat response (Ehlers and Hall 1998; Camejo et al. 2005).

In a well-planned breeding program, the knowledge of the genetic diversity among the species will facilitate the selection for key traits. The DNA-based Markers Assisted Selection (MAS) has several advantages over the traditional phenotypic selection and its potential benefits has been widely discussed (Melchinger 1990; Paterson et al. 1991; Young 1996; Mohan et al. 1997; Anderson 2003; Varshney and Tuberosa 2007). Molecular markers are a useful complement to morphological and physiological characterization because they are abundant and randomly distributed in the genome,

not influenced by tissue or environment and allow genotyping early in plant development. Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, defined here as a reduction of genetic diversity in time (Manifesto et al. 2001). Molecular makers can be very useful to better understand the genetic basis of phenotypic variability and might support the breeding process for the development of improved cultivars especially for traits which are difficult or expensive to measure, that exhibit low heritability and are expressed at late stages in plant development.

The present work aims to carry out a comprehensive phenotypic analysis of a durum wheat collection for the response to high-temperature followed by an association mapping study to detect Quantitative Trait Loci (QTLs) associated with heat tolerance. The response to chronic heat stress (Tashiro and Wardlaw 1989; Wardlaw et al. 1989; Yang et al. 2002a, b, c) and short-term heat shock (Tashiro and Wardlaw 1990; Plaut et al. 2004; Hays et al. 2007) has been studied in controlled environments where specific heat treatments may be applied.

Cell membrane stability (CMS; Sullivan 1972) is a trait often exploited as an indirect measurement for both heat and drought tolerance in various crops and it is considered a valuable index within breeding programs (Ibrahim and Quick 2001a, b; Ottaviano et al. 1991; Tripathy et al. 2000). Electrolyte leakage from leaf tissue after an “in vitro” or “in vivo” heat shock treatment at the seedling stage has been demonstrated to be associated with wheat performance at a range of heat-stressed field locations worldwide (Reynolds et al. 1994). Cell membrane stability is strictly related to denaturation of proteins and increments of unsaturated fatty acids that disrupt water, ion, and organic solute movement across membranes. Furthermore, disorders in thylakoid membranes trigger the physical separation of the chlorophyll light harvesting complex II from the PSII core complex, and disruption of PSII-mediated electron transfer (Ristic et al. 2008). The general protocol of CMS involves the application of stress to the leaf after it has been subjected to hardening, followed by the measurement of electrolyte leakage using the conductimetry method. When tissues are subjected to high temperature, electrical conductivity increases due to damage to the cell membrane and consequent increased permeability and leakage of ions out. Electrical conductivity has been already largely used as an index of membrane stability to identify heat-tolerant genotypes in wheat (Blum and Ebercon 1981) and for screening of heat-tolerant genotypes in different crops (Blum 1988).

Association mapping (AM), based on linkage disequilibrium, is a complementary strategy to traditional QTL mapping for describing associations between genotypes and phenotypes in crop plants (Ersoz et al. 2008; Sorrells and Yu 2009; Maccaferri et al. 2011).

In this research study, we investigated the results of an association mapping study aimed at mapping genes/QTLs for heat stress tolerance in durum wheat. In particular, the CMS trait under heat stress was chosen as an indicator trait for heat tolerance in a panel of durum wheat accessions well-suited for AM studies (Maccaferri et al. 2006, 2010 and 2011).

6.3 MATERIALS AND METHODS

6.3.1 Plant material

The panel of 183 elite accessions of durum wheat included cultivars and breeding lines developed in Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), Southwestern USA and Mexico that were released from the early 1970s up to the late 1990s (Appendix 1). The panel included also 'founder genotypes' used as parents in breeding programs throughout the Mediterranean Basin and at International CGIAR Centers (CIMMYT and ICARDA). The accessions were chosen according to their pedigree and highly related accessions were excluded. Accessions showing large differences in heading date were excluded to avoid possible bias of phenology in the interpretation of the results pertaining to the agronomic traits. A detailed phenotypic and molecular characterization of the panel was previously reported in Maccaferri et al. (2006, 2010 and 2011).

6.3.2 Heat stress response evaluation using cell membrane stability (CMS)

In this study, a panel of 183 elite accessions of durum wheat (Maccaferri et al. 2006) were germinated in petri dishes and vernalized for two weeks at 6°C. Seedlings were transplanted at four plants per pot filled with a substrate of Peat, sand and vermiculite in a 6:3:1 ratio. The experiment was conducted using a randomized complete block design with two replications in greenhouse environmental conditions. In the first step of experiment, 4-cm long leaf segments were sampled from leaves of four seedlings. The leaves were cut in 2-cm long segments, divided in a "control-sample" and a "treated-sample", washed in deionized water and placed in a plastic vial containing 15 ml of deionized water. The "treated-samples" were heat-treated in the incubator for 1 h at 45 °C.

After treatment, the samples (treated and control) were left at 4 °C for 24 h to allow the leakage of ions in the water solution. Ion leakage was then measured with a conductivity meter. After the measurements were taken, all samples were autoclaved for 15 min at 121°C and their conductance measured again. The CMS was calculated using the following formula (Blum and Ebercon 1981):

CMS (%) = [1-(T1/T2)/1–(C1/C2)] x100

where T is treatment samples, C is control samples, and 1 and 2 refer to the first and second readings of conductance, i.e., before and after autoclaving. This trait has been named ‘‘constitutive heat stress response‘‘ (CON).

In the second step of the experiment, heat stress was applied directly to plants at the booting stage by transferring the pots into the growth chamber at 37 °C for 24 h. Before applying the heat stress to plants, the “control-sample” was collected. Afterwards, the whole plant were heated for 5 h at 45 °C and the “treated-samples” collected. All samples were kept at 4 °C in darkness for 24 h. Solution conductance was then measured with a conductivity meter. After the measurements were taken, all samples were autoclaved for 15 min at 121°C and their conductance measured again. The CMS has been calculated using the formula previously described. This trait was named ‘‘acquired heat stress response‘‘ (AQU).

In the third step of experiment, samples collected from plants moved in growth chamber at 37 °C for 24 h (hardening), were heated in the incubator for 1 h at 45 °C. After the treatment all samples remained at the 4 °C in the darkness for 24 h. Solution conductance was then measured with a conductivity meter. After the measurements were taken, all samples were autoclaved for 15 min at 121°C and their conductance measured again. In this step of the experiment, we calculated CMS by using as control the value of the ions leakage measured on the samples used for the calculation of AQU_CMS before the heat-treatment. This trait was named ‘‘constitutive-acquired heat stress response‘‘ (CON_AQU).

6.3.3 Molecular profiling

In the present study, the SSR-based map (334 SSRs) reported in Maccaferri et al. (2011) after enrichment with DArT marker was utilized as reference for markers positions. In total, 957 markers (334 SSRs and 623 DArT markers) were used for the molecular profiling of the 183 accessions.

DArT markers were generated by Triticarte Pty. Ltd. (Canberra, Australia; http://www.triticarte.com.au). A durum wheat *PstI/TaqI* array v 2.0, containing 7,600 single DArT clones obtained as described in Mantovani et al. (2008), was used for genotyping the panel. The locus designation used by Triticarte Pty. Ltd. was adopted ('wPt', 'rPt' and 'tPt' loci corresponding to wheat, rye and triticale clones, respectively), and alleles at polymorphic loci were scored as hybridization positive (1) or negative (0).

Markers were ordered according to a consensus map developed at the University of Bologna in the framework of an international cooperation (Maccaferri et al. 2014). Four mapping populations, i.e. Kofa × Svevo (KS RIL population, Maccaferri et al. 2008), Colosseo × Lloyd (CL RIL, Mantovani et al. 2008), Meridiano × Claudio (MC RIL, Maccaferri et al. 2011) and Simeto × Levante (SL RIL, Maccaferri et al. unpublished) were developed by the University of Bologna in collaboration with Produttori Sementi Bologna SpA (Argelato, BO, Italy). Ten additional maps provided by international partners were used to assemble a common consensus map, used to order the markers available for genotyping the experimental materials herein presented (Maccaferri et al. 2014).

6.3.4 Statistical analysis and association mapping analysis

The genotypic and phenotypic coefficient of variation and heritability were calculated according to Singh and Chaudhary (1985). Heritability should be considered as being 'narrow sense' because the genetic variance included only the additive component and, possibly, the additive × additive epistatic interaction (Sanguineti et al. 2007).

For AM analysis, a total of 957 informative markers (*i.e.*, 334 SSR and 623 DArT markers) which was possible to project on the consensus linkage map were utilized. The presence of significant population structure in the panel had been previously shown by Maccaferri et al. (2011) with a combination of model- and distance-based analyses using the program STRUCTURE v. 2 (Pritchard et al. 2000). The population structure model optimum was identified in five hypothetical subgroups, that led to the *Q* matrix of membership coefficients of each accession to all subgroups (for details see Maccaferri et al. 2011).

A co-ancestry kinship (*K*) matrix was obtained for the mapped SSR and DArT markers by pairwise genetic similarity values (GS_{ij}) that were calculated for all accession pairs using the simple matching coefficient for multi-state markers. Linkage disequilibrium (LD) was estimated using the program

TASSEL, v. 2.1 (www. maizegenetics.net, Yu et al. 2006); *D'* and r^2 values are a function of the corresponding inter-marker distances and the comparison-wise significance was computed with 10,000 permutations. The r^2 LD value was estimated for intra-chromosomal loci and related to genetic distances between loci (cM). When all pairs of adjacent loci were in LD (arbitrarily set at $r^2 > 0.3$), this region was referred to as a LD block (Stich et al. 2005). Genome-wide scans for AM for heat stress response traits were conducted using the TASSEL program, ver. 5.1.0 (Bradbury et al. 2007). The 334 SSR and 623 DArT markers were tested for significance of marker-trait association under the fixed general linear model (GLM) including the *Q* population structure results as covariates (Q GLM), and the mixed linear model (MLM) including the *Q* population structure results plus the *K* kinship matrix (*Q*+*K* MLM).

For GLM analysis, besides the marker-wise association probability values, the experiment-wise association significance probability was obtained based on a permutation test implemented in TASSEL (10,000 permutations in total). The experiment-wise test provides a much more stringent threshold for significance as compared to the marker-wise test (Bradbury et al. 2007). Three significance levels of marker-trait association were considered, i.e. marker-wise at $P = 0.01$ (- log(P)) = 2.0) and $P = 0.001$ (- log10(P)) = 3.0) and experiment-wise at $P = 0.1$ (- log10(P)) = 4.0, Bonferroni's correction).

6.4 RESULTS AND DISCUSSION

Breeding for complex traits needs to take into account various factors, such as understanding the genetic, physiological and molecular bases of the traits, including interactions among the component traits and with the environments. Recently, progresses have been made in mapping and tagging many agriculturally important genes with molecular markers, which forms the basis for MAS in crop plants (Aneja et al. 2012). Molecular markers linked to the heat tolerance trait represent a more reliable tool for selecting heat tolerance genotypes in durum wheat breeding programs (Yang et al. 2002c; Kumar et al. 2013; Talukder et al. 2014). The purpose of this present study was the identification of QTLs in a set of elite durum wheat accessions in order to dissect the genetic control of heat-tolerance in durum wheat, using as index of resistance CMS at the seedling stage.

6.4.1 Phenotypic variation of the accessions' panel for heat stress response

Analysis of variance revealed highly significant differences (P <0.01) among durum wheat genotypes for all three measured traits (Table 1, Table 2). The experimental material showed a wide range of variation for the three traits. In detail, CON ranged from 1.8 to 133.5, with mean value of 39.26, AQU ranged from 60.7 to 133.6, with mean value of 98.2, and CON_AQU ranged from 18.7 to 115.5, with mean value of 71.2 (Table 1).

Coefficient of variation (CV) ranged from 4.8% for AQU, 6.1% for CON and 20.9% for CON_AQU. Heritability values ranged from 31% to 86% among traits and AQU and CON_AQU with the smallest and largest values, respectively (Table 1), indicating that the genetic to environmental control ratio was different among traits. As expected, the treatment carried out on the detached leaves (CON) showed higher heritability than the treatment on whole plants (AQU). It was also expected that the strongest treatment combining both protocols (CON_AQU) showed the highest heritability value among the three treatments.

Significant differences among wheat genotypes for CMS traits had been formerly observed (Shafeeq et al. 2006; Elshafei et al. 2013). Indeed, electrical conductivity has already been used as an index of membrane stability to identify heat-tolerant genotypes in wheat (Blum and Ebercon 1981) and for screening of heat-tolerant genotypes in different crops (Blum 1988). When tissues are subjected to high temperature, electrical conductivity increases due to damages to the cell membrane and consequent cytosolic leakage.

Histograms and cumulative distributions of the phenotypic data were inspected to assess the consistency of data and to investigate the complexity of the genetic control of the three heat response traits (Fig. 1 and 2). All the three traits, after square-root transformation, exhibited continuous variation with approximately normal distributions, suggesting a likely a polygenic quantitative control. Normality was confirmed with a Shapiro-Wilk test (data not shown).

6.4.2 Relationship between population structure and heat stress response

The genetic relationships among the accessions had been already investigated using both a genetic-similarity and a model-based Bayesian clustering method (Maccaferri et al. 2006, 2011; Letta et al. 2013). Results are reported in Fig. 3. Both methods pointed out that the minimum and optimum number of hypothetical well-distinct subgroups present in the panel were five. It was shown that the five subgroups corresponded to clearly distinct breeding lineages: 1) the ICARDA germplasm bred

for the dryland areas (subgroup S1); 2) the ICARDA germplasm bred for the temperate areas (subgroup S2); 3) the Italian and early ’70s CIMMYT germplasm (subgroup S3); 4) the late ’70s CIMMYT germplasm, widely adapted to Mediterranean conditions (subgroup S4); 5) the late ’80s, to early ’90s CIMMYT germplasm, with increased yield potential (subgroup S5). Based on the molecular assignment of each accession to the subgroup with the highest Bayesian probability, the five subgroups included 11, 55, 26, 56 and 35 accessions, respectively. The range values for the three heat response traits accounted for by population structure ranged from a minimum of 7.7% for CON to a maximum of 12.1% for AQU. These values can be considered rather low as compared to the reported influence of population structure on other important adaptive and agronomic traits in other species, such as maize and rice (Flint-Garcia et al. 2003; Zhao et al. 2011).

The mean of phenotypic variation for the constitutive, constitutive-acquired and acquired heat stress response of the 183 durum wheat accessions subdivided into the five main population structure groups are reported in Fig. 4. These values clearly show that all five sub-groups included accessions with a wide range of responses, thus indicating that all subgroups are equally informative and well-suited for AM purposes. For all three heat response traits, subgroups 1, 3 and 5 (CIMMYT germplasm of late 80s, early 90s) had the highest frequency of the phenotypic variation accessions. No significant relationship between phenotypic values of CMS traits and population structure was detected (data not shown), indicating that the variation observed here was not influenced by the coefficient of membership of the tested material to the five germplasm subgroups.

6.4.3 QTL analysis for heat stress response

QTL analysis results indicated the presence of QTLs for the investigated heat response traits in all chromosome groups (complete list of the QTLs are reported in Table 2). Overall, we identified 19 QTLs for CON, 5 for AQU and 10 for CON_AQU. Among these 34 QTLs, four QTLs (i.e., *QCON12_5A, QCON15_6A, QCON_AQU8_6B and QCON18_7B)* were significant at marker-wise significance level of $P < 0.001$ (-log10 > 3.0). Major QTLs (LOD ≥ 3) for CON were detected on chromosome 5A (R^2 value 8.0%), 6A (R^2 value 7.3%) and 7B (R^2 value 8.3%) and also for CON_AQU was detected only on chromosome 6B (R^2 value 7.7%). These four QTLs were therefore considered as high-confidence QTLs.

Table 3 lists the most representative durum varieties of the panel well-distributed to the five subgroups (Maccaferri et al. 2006) and some of the founders of the mapping populations available at the facilities of the University of Bologna. For each of the 16 genotypes the genetic contribution at the four major QTL is shown. Note that some genotypes from the CIMMYT or Italian breeding programs cumulate beneficial alleles at all four QTLs (Gallareta and Meridiano).

It is interesting that a higher number (nearly double) of QTLs were detected for CON heat response trait (heat shock applied to detached leaves) as compared to the two traits involving acquired responses (AQU and CON_AQU) measured on living plants pre-adapted to high temperatures. The reason for this result could be that the constitutive response on detached leaves has a less complex genetic basis and higher heritability than acquired resistance observed on intact plants.
Coincidence between QTLs for CON and CON_AQU was observed for a few QTLs, namely on chromosomes 5B (43.3 cM) and 7B (172.9 cM). Coincidence between QTLs for CON and AQU response was observed for only one QTL region (chromosome 3B, 86.3 cM).

6.5 CONCLUSIONS

Measurement of CMS is one such a technique that has been used to obtain a proxy index for both heat and drought tolerance in various crops. It is therefore a potentially interesting tool to help in the selection phase of breeding programs addressing increased heat tolerance. In this research study, we showed that assessing the response to heat stress based on cell membrane stability in durum wheat elite germplasm is feasible and informative in terms of QTL identification. The wide range of genetic variation found for the tested traits and the limited influence of population structure on the traits allow us to consider with confidence the possibility to use the QTL information identified in this study for subsequent validation of the physiological bases underlying the QTLs and their breeding value.

6.6 REFERENCES

Anderson JA (2003) Plant genomics and its impact on wheat breeding. In: Plant molecular breeding (H.J.Newbury, Ed.), Blackwell Publ., Boca Raton, 184-215

Aneja B, Yadav NR, Chawla V, Yadav RC (2012) Sequence-related amplified polymorphism (SRAP) molecular marker system and its applications in crop improvement. Mol Breeding 30:1635–1648

Belaid A (2000) Durum wheat in WANA: production, trade and gains from technological change. Proceedings of the seminar 'Durum wheat improvement in the Mediterranean region: new challenges. Zaragoza, Spain, 12–14 April 2000. Options Mediterraneennes, Serie A, Seminaires Mediterraneens, 40, 35–49

Blum, A (1988) Plant breeding for stress environments. CRC Press, Inc., Boca Raton, Florida, pp. 223

Blum A, Ebercon A (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science 21: 43-47

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635. doi:10.1093/bioinformatics/btm308

Camejo D, Rodríguez P, Morales MA, Dell'amico JM, Torrecillas A, Alarcón JJ (2005) High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J. Plant Physiol. 162: 281-289

Deryng D, Conway D, Ramankutty N, Price J, Warren R (2014) Global crop yield response to extreme heat stress under multiple climate change futures Environ. Res. Lett. 9-034011

Ehlers JD, Hall AE (1998) Heat tolerance of contrasting cowpea lines in short and long days. Field Crops Res. 55: 11-21

Elshafei AA, Saleh M, Al-Doss AA, Moustafa KA, Al-Qurainy FH, Barakat MN (2013) Identification of new SRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stressed condition. Aust. J. Crop Sci. 7: 887–893

Ersoz ES, Yu J, Buckler ES (2007) Applications of linkage disequilibrium and association mapping. In: Varshney RK, Tuberosa R (eds) Genomics-assisted crop improvementvol 1: genomics

approaches and platforms, vol 1. Springer, New York, pp 97–120

Flint-Garcia SA, Thronsberry JM, Buckler IV ES (2003) Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol. 2003. 54:357–74

Gourdji SM, Sibley AM, Lobell DB (2013) Global crop exposure to critical high temperatures in the reproductive period: historical trends and future projections Environ. Res. Lett. 8-024041

Habash DZ, Kehel Z, Nachit M (2009) Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J Exp Bot 60: 2805–2815

Hays DB, Do JH, Mason RE, Morgan G, Finlayson SA (2007) Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Science 172:1113-1123

Kumar S, Kumari P, Kumar U, Grover M, Kumar Singh A, Singh R, Sengar RS (2013) Molecular approaches for designing heat tolerant wheat. J. Plant Biochem. Biotechnol. DOI 10.1007/s13562-013-0229-3

Ibrahim A, Quick JS (2001a) Heritability of heat tolerance in winter and spring wheat. Crop Sci. 41:1401–1405.

Ibrahim A, Quick JS (2001b) Genetic control of high temperature tolerance in wheat as measured by membrane thermal stability. Crop Sci. 41:1405–1407

Letta T, Maccaferri M, Ammar K, Badebo A, Ricci A, Crossa J, Tuberosa R (2013) Searching for novel sources of field resistance to Ug99 and Ethiopian stem rust races in durum wheat via association mapping. Theor. Appl. Genet. DOI: 10.1007/s00122-013-2050-8

Lobell DB, Sibley A, Ortiz-Monasterio JI (2012) Extreme heat effects on wheat senescence in India Nat. Clim. Change 2, 186–9

Maccaferri M, Sanguineti MC, Natoli V, Ortega JLA, Ben Salem M, Bort J, Chenenaoui C, De Ambrogio E, del Moral LG, De Montis A, El-Ahmed A, Maalouf F, Machlab H, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Tuberosa R (2006) A panel of elite accessions of durum wheat (Triticum durum Desf.) suitable for association mapping studies. Plant Gen Res 4: 79–85

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Ben Salem M, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008)

Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178: 489-511

Maccaferri M, Sanguineti MC, Mantovani P, Demontis A, Massi A, Ammar K, Kolmer J, Czembor J, Ezrati S, Tuberosa R (2010) Association mapping of leaf rust response in durum wheat. Mol Breeding 26: 189-228

Maccaferri M, Sanguineti MC, del Moral LFG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2011) Association mapping in durum wheat grown across a broad range of water regimes and yield potential. J Exp Bot 62: 409-438

Maccaferri M, Cane MA, Salvi S, Noli E, Colalongo C,Massi A, Clarke F, Pozniak C, Korol A, Fahima T, Dubcovsky J, Xu S, Karsai I, Knox R, Clarke J, Tuberosa R (2014) A consensus framework map of durum wheat (Triticum durum Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. BMC Genomics 15:873

Manifesto MM, Schlatter A, Hopp HE, Suarez EY, Dubcovsky J (2001) Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci. 41: 682-690

Mantovani P, Maccaferri M, Sanguineti MC, Tuberosa R, Catizone I, Wenzl P, Thomson B, Carling J, Huttner E, DeAmbrogio E, Kilian A (2008) An integrated DArT-SSR linkage map of durum wheat. Mol Breed 22: 629–648

Marti J, Slafer GA (2014) Bread and durum wheat yields under a wide range of environmental conditions. Field Crops Research 156, 258–271

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104: 1-19

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker assisted selection in crop plants. Mol Breed 3:87-93

Morancho J (2000) Production and marketing of durum wheat worldwide. Proceedings of the seminar ‘Durum wheat improvement in the Mediterranean region: new challenges. Zaragoza, Spain, 12–14 April 2000. Options Mediterraneennes, Serie A, Seminaires Mediterraneens, 40, 35–49

Ottaviano E, Gorla MS, Pe E, Frova C (1991) Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. Theor. Appl. Genet. 81:713–719

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv. Agron. 46:39-90

Plaut Z, Butow BJ, Blumenthal CS, Wrigley CW (2004) Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated temperature. Field Crops Research 86:185-198

Pradhan GP, Vara Prasad PV, Fritz AK, Kirkham MB, Gill BS (2012) Effects of drought and high temperature stress on synthetic hexaploid wheat. Functional Plant Biology 39(3) 190-198

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association mapping in structured populations. Am J Hum Genet 67:170–181

Reynolds M, Balota M, Delgado M, Amani I, Fischer R (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Funct Plant Biol 21: 717–730

Ristic Z, Bukovnik U, Vara Prasad PV, West M (2008) A model for prediction of heat stability of photosynthetic membranes. Crop Sci 48: 1513–1522

Sanguineti MC, Li S, Maccaferri M, Corneti S, Rotondo F, Chiari T, Tuberosa R (2007) Genetic dissection of seminal root architecture in elite durum wheat germplasm. Ann Appl Biol 151:291–305. doi:10.1111/j.1744-7348.2007. 00198.x

Shafeeq S, Rahman M, Zafar Y (2006) Genetic variability of different wheat (Triticum aestivum L.). Pak. J. Bot. 38: 1671–1678

Singh RK, Chaudhary BD (1985) Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers Ludhiana, New Delhi. pp. 102-164

Sorrells ME, Yu J (2009) Linkage disequilibrium and association mapping in the Triticeae. Genet Genomics Triticeae 7:655–683. doi:10.1007/978-0-387-77489-3-22

Stich B, Melchinger AE, Frisch M, Maurer HP, Heckenberger M, Reif JC (2005) Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theor Appl Genet 111:723–730

Sullivan CY (1972) Mechanism of heat and drought resistance in grain sorghum and methods of measurement. In: Rao NGP, House LR, editors. Sorghum in the seventies. New Delhi, India: Oxford & IBH publ Co.; p. 247–64

Talukder S, Babar M, Vijayalakshmi K, Poland J, Prasad P, Bowden R, Fritz A (2014) Mapping

QTL for the traits associated with heat tolerance in wheat (Triticum aestivum L.). BMC Genet. 15:97

Tashiro T, Wardlaw IF (1989) A comparison of the effect of high-temperature on grain development in wheat and rice. Annals of Botany 64:59-65

Tashiro T, Wardlaw IF (1990) The response to high-temperature shock and humidity changes prior to and during the early stages of grain development in wheat. Australian Journal of Plant Physiology 17:551-561

Tripathy JN, Zhang J, Robin S, Nguyen HT (2000) QTLs for cell-membrane stability mapped in rice (Oryza sativa L.) under drought stress. Theor. Appl. Genet.100:1197–1202

Varshney RK, Tuberosa R (2007) Application of linkage disequilibrium and association mapping in crop plants. Genomics Approaches and Platforms, Vol 1: 97-119

Wahid A, Gelani S, Ashraf M, Foolad M (2007) Heat tolerance: an overview. Environ. Exp. Bot. 61, 199–223

Wardlaw IF, Dawson IA, Munibi P, Fewster R (1989) The tolerance of wheat to hightemperatures during reproductive growth: 1. survey procedures and general response patterns. Australian Journal of Agricultural Research 40:1-13

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479-501

Yang J, Sears RG, Gill BS, Paulsen GM (2002a) Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock stresses - Utilization of assimilate sources by wheat under heat stresses. Euphytica 125:179-188

Yang J, Sears RG, Gill BS, Paulsen GM (2002b) Growth and senescence characteristics associated with tolerance of wheat-alien amphiploids to high temperature under controlled conditions. Euphytica 126:185-193

Yang J, Sears RG, Gill BS, Paulsen GM (2002c) Quantitative and molecular characterization of heat tolerance in hexaploid wheat. Euphytica 126:275-282

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208. doi:10.1038/ ng1702

Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nature communications. DOI: 10.1038/ncomms1467

FIGURES

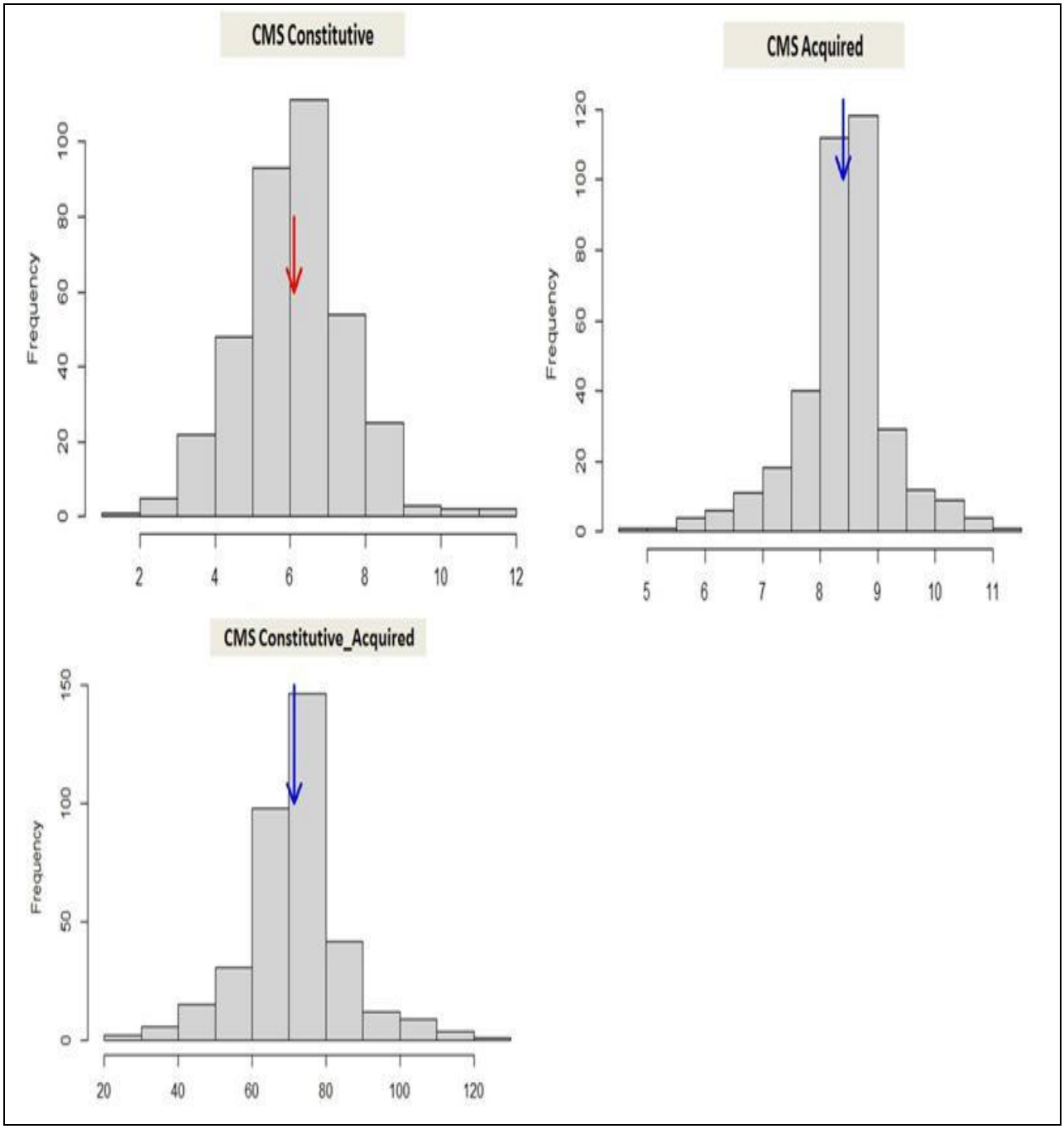

Fig. 1 Histogram distribution for the constitutive, constitutive-acquired and acquired heat stress response of the 183 durum wheat accessions

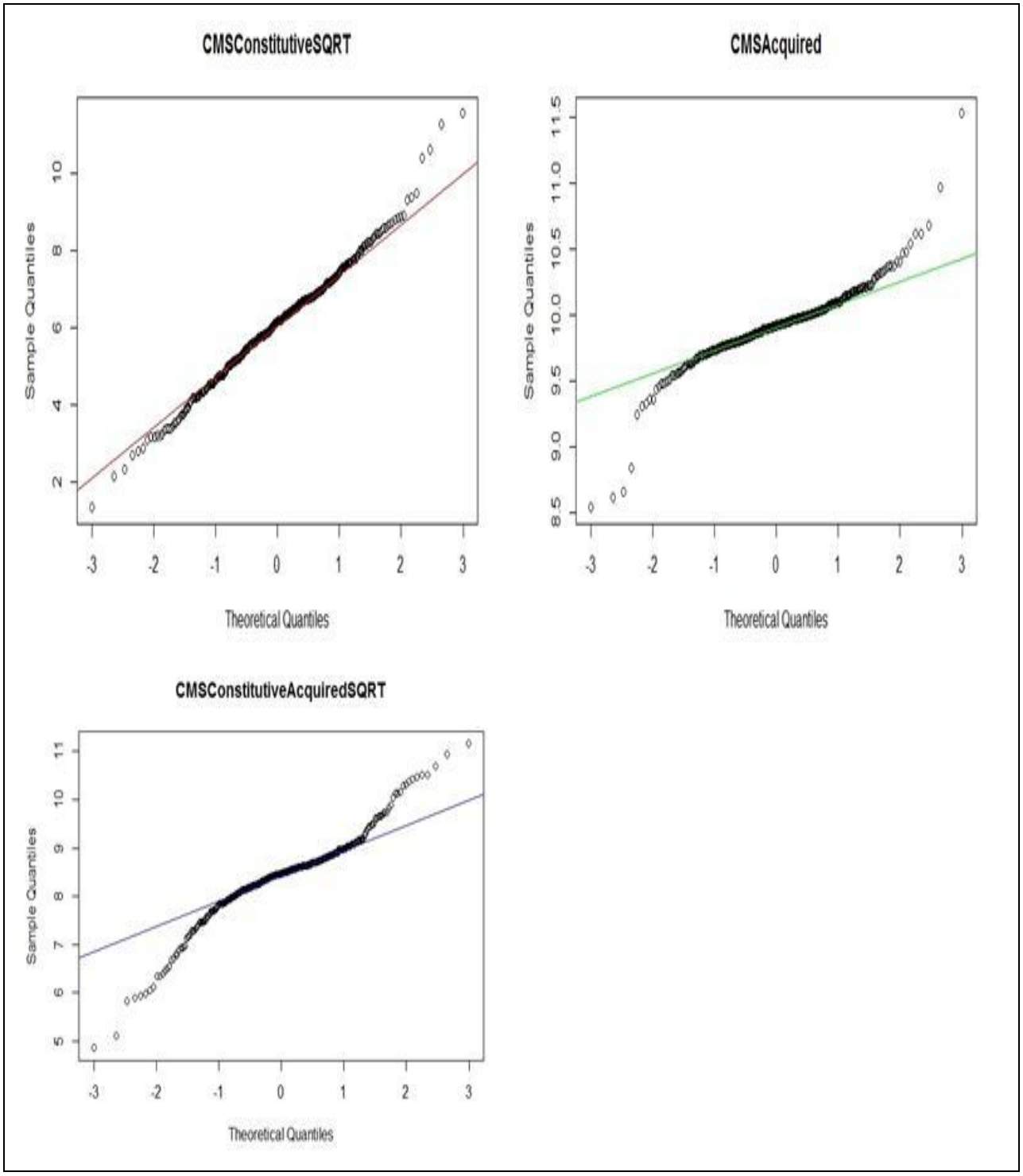

Fig. 2 Cumulative distribution for the constitutive, constitutive-acquired and acquired heat stress response of the 183 durum wheat accessions

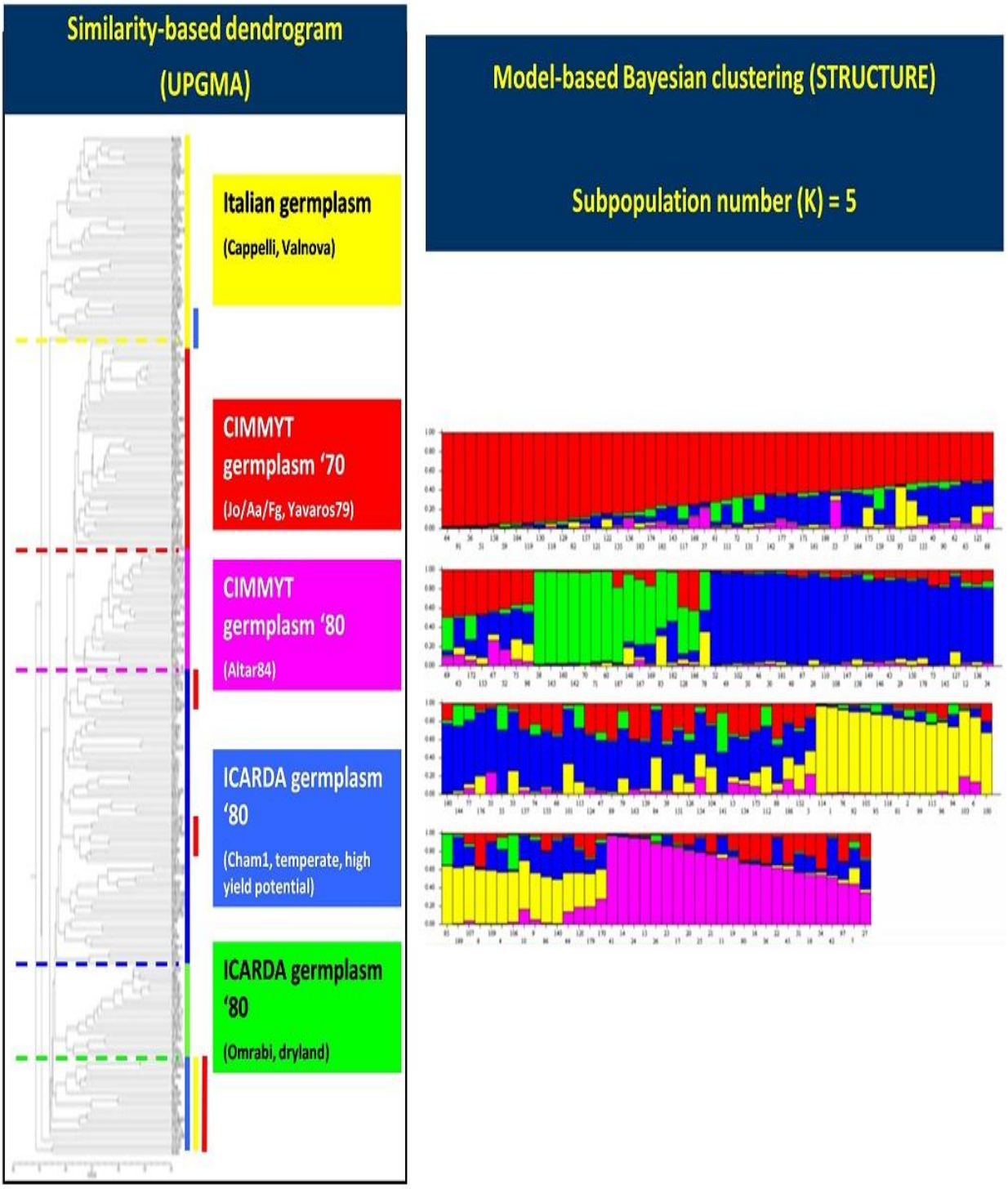

Fig. 3 Population structure investigated by both distance and model based Bayesian analysis (Figures taken from Maccaferri et al. 2006)

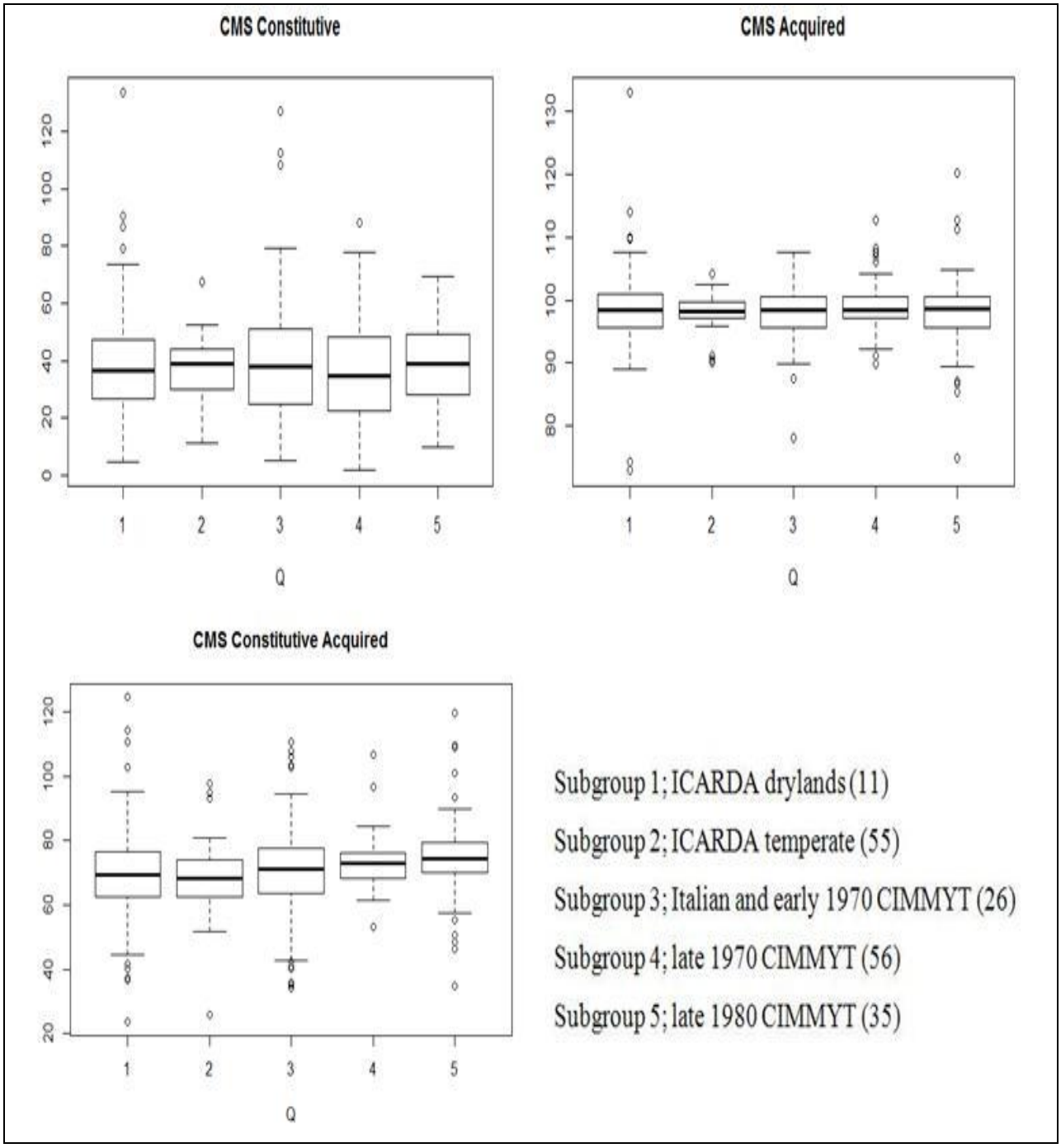

Fig. 4 The mean of phenotypic variation for the constitutive, constitutive-acquired and acquired heat stress response of the 183 durum wheat accessions subdivided into the five main population structure groups

TABLES

Table 1 Mean, minimum, maximum values for the constitutive, constitutive-acquired and acquired heat stress response of the 183 durum wheat accessions. Coefficient of variation and heritability values for each trait are reported

Trait	CON	AQU	CON_ AQU
Mean	39.26	98.2	71.2
Max	133.5	133.6	115.5
Min	1.8	60.7	18.7
P accessions [a]	**	**	**
P replicates [b]	ns	ns	**
CV (%)	6.1	4.8	20.9
h^2 (%)	59	31	86

[a] Significance of the difference between accessions

[b] Significance of the difference between replicates. ns = non significant, ** P<0.01

Table 2 Complete list of the QTLs identified with significant concurrent effects for the constitutive, constitutive-acquired and acquired heat stress response

QTL	Trait	Chr.	Marker	Peak (cM)	P(−log10) *	R^2 %	Effect	Effect% of mean
QCON1_1A	CON.	1A	wmc95-1A1-a1	18.6	2.09	3.92	-12.10	30.81
QCON_AQU1_1B	CON_AQU.	1B	gpw3013-1B-a4	26.7	2.71	5.44	9.57	13.44
QCON2_1B	CON.	1B	wmc85-1B-a2	0.5	2.13	4.02	-10.77	27.42
QCON3_1B	CON.	1B	wmc500-1B-a4	20.8	2.06	3.87	11.81	30.07
QCON_AQU2_2A	CON_AQU.	2A	wmc658-2A-a1	17.5	2.72	5.47	7.20	10.11
QCON_AQU3_2B	CON_AQU.	2B	wmc361-2B-a3	225.7	1.67	2.98	-6.59	9.26
QCON4_2B	CON.	2B	wPt-5513-2B	45.2	1.47	2.51	-5.58	14.22
QCON5_2B	CON.	2B	barc55-2B-a6	65.5	2.88	5.84	-4.08	10.38
QCON6_3A	CON.	3A	wPt-2810-3A	2.1	2.22	4.24	-9.43	24.02
QAQU1_3A	AQU.	3A	barc1113-3A-a1	146.4	2.22	4.50	-2.44	2.49
QCON_AQU4_3B	CON_AQU.	3B	wPt-3536-3B	17.2	2.27	4.38	10.35	14.54
QCON_AQU5_3B	CON_AQU.	3B	ksm45-3B-a4	44.5	2.85	5.80	-11.78	16.54
QCON7_3B	CON.	3B	rPt-5396-3B	86.3	2.59	5.13	9.03	23.01
QCON8_3B	CON.	3B	wmc418-3B-a4	113.8	2.71	5.42	13.39	34.10
QAQU2_3B	AQU.	3B	wPt-9432-3B	87.8	2.41	4.98	2.91	2.97
QAQU3_3B	AQU.	3B	wPt-7145-3B	147.3	2.01	3.97	-2.17	2.21
QAQU4_3B	AQU.	3B	wPt-8959-3B	204.1	1.55	2.85	2.48	2.52
QCON_AQU6_4A	CON_AQU.	4A	wmc617-4A-a1	37.9	2.01	3.75	10.65	14.96
QCON9_4A	CON.	4A	barc155-4A-a3	54	2.01	3.74	8.90	22.67
QCON10_4A	CON.	4A	wPt-7289-4A	96.1	1.87	3.43	-9.05	23.06
QAQU5_4A	AQU.	4A	wPt-1262-4A	88.4	1.73	3.27	-3.03	3.08
QCON11_4B	CON.	4B	wPt-8543-4B	66.9	2.80	5.64	-12.05	30.69
QCON12_5A	CON.	5A	gwm126-5A-a3	155.1	3.73	8.00	-11.50	29.28
QCON_AQU7_5B	CON_AQU.	5B	barc74-5B-a4	43.3	2.46	4.84	5.62	7.89
QCON13_5B	CON.	5B	barc74-5B-a4	43.3	2.11	3.99	-2.90	7.39
QCON14_5B	CON.	5B	wPt-5604-5B	111.5	1.64	2.90	17.49	44.55
QCON15_6A	CON.	6A	gwm1089-6A-a6	131	3.46	7.30	14.53	37.00
QCON_AQU8_6B	CON_AQU.	6B	wPt-8336-6B	5.1	3.59	7.67	7.00	9.83
QCON_AQU9_6B	CON_AQU.	6B	wPt-1325-6B	157.9	2.06	3.87	5.97	8.38
QCON16_7A	CON.	7A	wPt-7053-7A	172.1	1.74	3.12	8.01	20.40
QCON_AQU10_7B	CON_AQU.	7B	gwm611-7B2-a4	172.9	2.63	5.25	9.52	13.37
QCON17_7B	CON.	7B	gwm3019-7B-a2	62.8	2.13	4.02	-15.59	39.72
QCON18_7B	CON.	7B	wmc526-7Ba-a3	190.8	3.85	8.32	-15.56	39.62
QCON19_7B	CON.	7B	gwm611-7B2-a8	172.9	2.25	4.57	2.13	2.17

*QTLs with (−log10) <2 are considered as low- confidence QTLs

Table 3 Results of four major QTLs (LOD ≥ 3) were detected for the constitutive, constitutive-acquired heat stress response among the 183 tested durum wheat accessions

	QCON12_5A	**QCON15_6A**	**QCON18_7B**	**QCON_AQU8_6B**
P(−log10) >3.0	3.73	3.46	3.85	3.59
Effect	-11.50 (A)	14.53 (T)	-15.56 (T)	7.0 (A)
CAPEITI	A(-)	T(+)	T(-)	T(-)
CAPPELLI	T(+)	T(+)	T(-)	T(-)
CHAM-1	A(-)	A(-)	T(-)	T(-)
CLAUDIO	A(-)	T(+)	T(-)	T(-)
COLOSSEO	T(+)	T(+)	T(-)	A(+)
CRESO	T(+)	T(+)	T(-)	A(+)
GALLARETA	T(+)	T(+)	A(+)	A(+)
HAURANI	A(-)	T(+)	T(-)	T(-)
IRIDE	A(-)	T(+)	T(-)	A(+)
KARIM	T(+)	T(+)	T(-)	A(+)
KOFA	A(-)	T(+)	T(-)	T(-)
MERIDIANO	T(+)	T(+)	A(+)	A(+)
MEXICALI 75	A(-)	A(-)	T(-)	A(+)
MOHAWK	T(+)	T(+)	T(-)	T(-)
SVEVO	T(+)	A(-)	A(+)	A(+)
VALNOVA	T(+)	A(-)	T(-)	A(+)

Appendix

Appendix 1 Name and origin of durum association panel used in this study

Accession code	Accession name	ORIGIN
IDUWUE-002	CANNIZZO	ITALY
IDUWUE-003	CLAUDIO	ITALY
IDUWUE-004	LESINA	ITALY
IDUWUE-005	MERIDIANO	ITALY
IDUWUE-006	MONGIBELLO	ITALY
IDUWUE-007	NORBA	ITALY
IDUWUE-008	PIETRAFITTA	ITALY
IDUWUE-010	TORREBIANCA	ITALY
IDUWUE-011	BISU_1/PATKA_3	CIMMYT
IDUWUE-012	CMH82A.1062/3/GGOVZ394//SBA81/PLC/4/AAZ_1/C REX/5/HUI//CIT71/CII	CIMMYT
IDUWUE-013	DUKEM/3/RUFF/FGO//YAV79	CIMMYT
IDUWUE-015	KULRENGI-BALIKCIL_8	CIMMYT
IDUWUE-016	PLATA_16	CIMMYT
IDUWUE-017	PORTO_5	CIMMYT
IDUWUE-018	ROK/FGO//STIL/3/BISU_1	CIMMYT
IDUWUE-020	ACUATICO/YAZI_1	CIMMYT
IDUWUE-021	FOCHA_1/5*ALAS	CIMMYT
IDUWUE-023	BUSHEN_4/TARRO_2//BUSHEN_4	CIMMYT
IDUWUE-024	GS/CRA//SBA81/3/HO/4/MEXI_1/5/MEMO/6/2*ALT AR 84	CIMMYT
IDUWUE-025	RASCON_37/2*TARRO_2	CIMMYT
IDUWUE-027	SRN_3/AJAIA_15//DUKEM_1/3/DION_2	CIMMYT
IDUWUE-028	ALDEANO	IRTA-SPAIN
IDUWUE-029	ARIESOL	IRTA-SPAIN
IDUWUE-030	ARTENA	IRTA-SPAIN
IDUWUE-031	ASTIGI	IRTA-SPAIN
IDUWUE-032	BOABDIL	IRTA-SPAIN
IDUWUE-033	BOLENGA	IRTA-SPAIN
IDUWUE-034	BOLIDO	IRTA-SPAIN
IDUWUE-035	BOLO	IRTA-SPAIN
IDUWUE-036	BOMBASI	IRTA-SPAIN
IDUWUE-037	BORLI	IRTA-SPAIN
IDUWUE-038	CANYON	IRTA-SPAIN
IDUWUE-039	DURCAL	IRTA-SPAIN
IDUWUE-040	DUROI	IRTA-SPAIN
IDUWUE-041	GALLARETA	IRTA-SPAIN
IDUWUE-042	ILLORA	IRTA-SPAIN
IDUWUE-044	SENADUR	IRTA-SPAIN
IDUWUE-045	SULA	IRTA-SPAIN
IDUWUE-047	NASSIRA (MOROCCO_1805)	INRA-MOROCCO
IDUWUE-048	CHAOUI (MOROCCO_1807)	INRA-MOROCCO
IDUWUE-049	AMRIA (MOROCCO_1808)	INRA-MOROCCO
IDUWUE-050	MAROUANE (MOROCCO_1809)	INRA-MOROCCO
IDUWUE-053	JAWHAR	INRA-MOROCCO
IDUWUE-054	MARJANA	INRA-MOROCCO
IDUWUE-055	MARZAK	INRA-MOROCCO

Appendix 1 Continued…

Accession code	Accession name	ORIGIN
IDUWUE-056	OURGH	INRA-MOROCCO
IDUWUE-057	TAREK	INRA-MOROCCO
IDUWUE-060	AWALBIT	ICARDA
IDUWUE-061	BCR/3/CHAM_1//GTA/STR	ICARDA
IDUWUE-062	CHHB88/DERAA	ICARDA
IDUWUE-063	CHACAN	ICARDA
IDUWUE-064	KARIM	ICARDA
IDUWUE-065	HML/CHHB88	ICARDA
IDUWUE-066	KRS/HCN	ICARDA
IDUWUE-067	MURLAGOST-3	ICARDA
IDUWUE-068	MOULSABIL_2	ICARDA
IDUWUE-069	OMBAR	ICARDA
IDUWUE-071	MRB589_5	ICARDA
IDUWUE-072	QUADALETE//ERP/MAL/3/UNKNOWN(VSGI,ODESSA)	ICARDA
IDUWUE-073	SEBAH	ICARDA
IDUWUE-074	STOJOCRI_3	ICARDA
IDUWUE-075	ZEINA_1	ICARDA
IDUWUE-076	ANTON	ICARDA
IDUWUE-077	APPIO	ITALY
IDUWUE-079	ARCANGELO	ITALY
IDUWUE-080	ARCOBALENO	ITALY
IDUWUE-081	BRAVADUR	DESERT
IDUWUE-082	BRONTE	ITALY
IDUWUE-083	CAPEITI 8	ITALY
IDUWUE-084	CAPPELLI	ITALY
IDUWUE-085	CICCIO	ITALY
IDUWUE-086	COLORADO-DW	DESERT
IDUWUE-087	COLOSSEO	ITALY
IDUWUE-088	CORTEZ	DESERT
IDUWUE-089	CRESO	ITALY
IDUWUE-090	DON PEDRO	ITALY
IDUWUE-091	DUILIO	ITALY
IDUWUE-093	FLAMINIO	ITALY
IDUWUE-094	FORTORE	ITALY
IDUWUE-095	GARGANO	ITALY
IDUWUE-096	GRAZIA	ITALY
IDUWUE-097	IRIDE	ITALY
IDUWUE-098	ITALO	ITALY
IDUWUE-099	IXOS	ITALY
IDUWUE-100	KRONOS	DESERT
IDUWUE-102	MESSAPIA	ITALY
IDUWUE-103	MEXICALI 75	ITALY
IDUWUE-104	MOHAWK	ITALY
IDUWUE-105	OFANTO	ITALY
IDUWUE-106	PLATANI	ITALY
IDUWUE-107	PLINIO	ITALY
IDUWUE-108	PRODURA	ITALY
IDUWUE-109	REVA	ITALY
IDUWUE-110	ROQUENO	ITALY

Appendix 1 Continued…

Accession code	Accession name	ORIGIN
IDUWUE-111	SVEVO	ITALY
IDUWUE-112	TRINAKRIA	ITALY
IDUWUE-113	VALBELICE	ITALY
IDUWUE-114	VALNOVA	ITALY
IDUWUE-116	WESTBRED 881	DESERT
IDUWUE-117	WESTBRED TURBO	DESERT
IDUWUE-118	AGHRASS_1	ICARDA
IDUWUE-119	AINZEN_1	ICARDA
IDUWUE-120	ANGRE	ICARDA
IDUWUE-121	AMEDAKUL-1	ICARDA
IDUWUE-122	AMMAR-1	ICARDA
IDUWUE-123	ARISLAHN-5	ICARDA
IDUWUE-124	ATLAST-1	ICARDA
IDUWUE-125	AUS1	ICARDA
IDUWUE-126	AWALI_1	ICARDA
IDUWUE-127	RADIOSO	ITALY
IDUWUE-128	AZEGHAR_2	ICARDA
IDUWUE-130	BICRE	ICARDA
IDUWUE-131	BICREDERAA_1	ICARDA
IDUWUE-132	BIGOST-1	ICARDA
IDUWUE-133	BELIKH 2	ICARDA
IDUWUE-134	BRACHOUA	ICARDA
IDUWUE-135	CHAHBA88	ICARDA
IDUWUE-136	CHAM_1	ICARDA
IDUWUE-137	DERAA	ICARDA
IDUWUE-139	GEROMTEL-1	ICARDA
IDUWUE-140	GEZIRA-17	ICARDA
IDUWUE-141	GIDARA_2	ICARDA
IDUWUE-142	GUEROU_1	ICARDA
IDUWUE-144	HAURANI	ICARDA
IDUWUE-145	HEIDER	ICARDA
IDUWUE-146	OSL_1/4/BUC/CHRC//PRL/3/PVN/5/HEL/3/YAV/COR M//SHWA	ICARDA
IDUWUE-147	SEBOU	ICARDA
IDUWUE-148	BLK2//134XS-69-186/368-1/3/MRB589_5/4/ALBT_3	ICARDA
IDUWUE-149	ARIC31708.70/3/BO-DW//CDECH/BR-DW/4/CIT71/GT	ICARDA
IDUWUE-150	JORDAN	ICARDA
IDUWUE-151	KABIR 1	ICARDA
IDUWUE-153	KHABUR_1	ICARDA
IDUWUE-154	KORIFLA	ICARDA
IDUWUE-155	LAGONIL-2	ICARDA
IDUWUE-156	LAHN	ICARDA
IDUWUE-157	LOUKOS_1	ICARDA
IDUWUE-158	MAAMOURI-1	ICARDA
IDUWUE-159	MARSYR-1	ICARDA
IDUWUE-160	MASSARA_1	ICARDA
IDUWUE-161	MIKI-1	ICARDA
IDUWUE-163	MURLAGOST-1	ICARDA
IDUWUE-164	NILE	ICARDA
IDUWUE-166	OMGENIL_3	ICARDA
IDUWUE-167	OMLAHN-3	ICARDA

Appendix 1 Continued….

Accession code	Accession name	ORIGIN
IDUWUE-168	OMRUF-2	ICARDA
IDUWUE-169	OMSNIMA-1	ICARDA
IDUWUE-170	ORONTE 1	ICARDA
IDUWUE-171	OTB-6	ICARDA
IDUWUE-172	OUASERL_1	ICARDA
IDUWUE-173	OUASLAHN-1	ICARDA
IDUWUE-175	QUABRACH-1	ICARDA
IDUWUE-176	QUADALETE	ICARDA
IDUWUE-177	RAZZAK	INRAT
IDUWUE-178	SAADA3/DDS//MTL-1	ICARDA
IDUWUE-179	SAJUR	ICARDA
IDUWUE-181	SHABHA	ICARDA
IDUWUE-182	TELSET_5	ICARDA
IDUWUE-183	TENSIFT_1	ICARDA
IDUWUE-184	TERBOL 97_3	ICARDA
IDUWUE-185	TUNSYR-1	ICARDA
IDUWUE-186	WADALMEZ_1	ICARDA
IDUWUE-187	YOUNES-1	ICARDA
IDUWUE-188	YOUSEF_1	ICARDA
IDUWUE-189	KOFA	ITALY
CIMMYT-251	1A.1D 5+10-6/3*MOJO//RCOL	CIMMYT
CIMMYT-252	SOOTY_9/RASCON_37	CIMMYT
CIMMYT-253	STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-254	SOMAT_4/INTER_8	CIMMYT
CIMMYT-255	CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-256	MALMUK_1//LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)	CIMMYT
CIMMYT-257	1A.1D 5+10-6/2*WB881//1A.1D 5+10-6/3*MOJO/3/BISU_1/PATKA_3	CIMMYT
CIMMYT-258	HESSIAN-F_2/3/STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-259	AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMAT_3/4/SOOTY_9/RASCON_37	CIMMYT
CIMMYT-260	USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9	CIMMYT
CIMMYT-261	CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	CIMMYT
CIMMYT-262	GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/3/YAV_1/GEDIZ/6/SOMBRA_20/7/STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-263	VANRRIKSE_6.2//1A-1D 2+12-5/3*WB881	CIMMYT
CIMMYT-264	RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN	CIMMYT
CIMMYT-265	PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT	CIMMYT
CIMMYT-266	EUDO//CHEN_1/TEZ/3/TANTLO_1/4/PLATA_6/GREEN_17	CIMMYT
CIMMYT-267	ROLA_5/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/MALMUK_1/SERRATOR_1	CIMMYT
CIMMYT-268	ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1	CIMMYT
CIMMYT-269	SOMAT_3/PHAX_1//TILO_1/LOTUS_4	CIMMYT